HEMATOLOGICAL AND GENETIC PROFILE OF MYELOPROLIFERATIVE NEOPLASMS

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Submitted By

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I hereby declare that the thesis entitled "**HEMATOLOGICAL AND GENETIC PROFILE OF MYELOPROLIFERATIVE NEOPLASMS**" submitted towards partial fulfillment of the requirements for the award of **MD in Pathology** degree to BFUHS is a compilation of original work carried out by me under the supervision of **Dr. Bhavna Malhotra** , Professor, Department of Pathology, **Dr. Vikram Narang**, Associate Professor, Department of Pathology and **Dr. Davinder Paul**, Assistant Professor, Department of Oncology. No part of this thesis has formed the basis for the award of any degree previously.

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Dedicated

to

my Family

&

Teachers

LIST OF ABBREVIATIONS

CML	Chronic myeloid leukemia
EDTA	Ethylenediaminetetraacetic acid
ET	Essential thrombocythemia
FISH	Fluorescent insitu hybridization
g/dl	Gram per deciliter
Hb	Hemoglobin
H&E	Hematoxylin and Eosin
MGG	May Grunwwald- Giemsa
MPN	Myeloproliferative neoplasm
MT	Masson trichrome
nRBC	Nucleated red blood cells
NGS	Next generation sequencing
PBF	Peripheral blood film
PMF	Primary myelofibrosis
PV	Polycythemia vera
RBC	Red blood cells
RT-PCR	Reverse transcriptase polymerase chain reaction

ABSTRACT

Introduction: Chronic myeloproliferative neoplasms are clonal stem cell disorders encompassing a very heterogeneous complex of different entities which are defined by distinct clinical and cytomorphological phenotypes and known genetic features. They are characterized by increased and effective proliferation of one to three hematopoietic cell lineages in the bone marrow associated to increased peripheral blood parameters.

Aims and objectives: To correlate the genetic profile of patients with myeloproliferative neoplasm with hemograms, bone marrow aspirations and bone marrow biopsy findings.

Materials and methods: The present five year study was conducted in the Department of Pathology, Dayanand Medical College and Hospital. During this period all the cases of myeloproliferative neoplasms wereanalyzedwith respect to their clinical, hematologicalprofile and molecular genetics.

Results: Of 138 cases included majority of patients were in age group of 41 to 50 years [35 cases, 25.4%] with slight male predominance was noted with M:F was 1.3:1.CML [105 /138, 76.3%] was commonest followed by ET 13 cases [9.42%] , PV 10 cases [7.19%] and PMF 10 cases [7.19%] .

Conclusion: Most MPNs exhibit age-matched hypercellularity of the bone marrow, effective haematopoietic maturation, and a varied increase in the numbers of granulocytic, erythrocytic, and megakaryocytic lineages in peripheral blood and bone marrow. Splenomegaly and hepatomegaly are typical symptoms of abnormal haematopoietic progenitor cell sequestration.

Keywords: myeloproliferative neoplasm, molecular genetics, hematological profile

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(Dr. Jasleen Kaur)

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INTRODUCTION

Chronic myeloproliferative neoplasms are clonal stem cell disorder that cover a very varied group of various entities characterised by discrete clinical and cytomorphological characteristics as well as known genetic markers. They are distinguished by increased proliferation of one to three hematopoietic cell lineages in the bone marrow, as well as elevated peripheral blood parameters. These stem cell disorders are categorised as BCR-ABL positive CML or BCR-ABL negative polycythemia vera [PV], essential thrombocythemia[ET], and primary myelofibrosis [PMF]. These BCR-ABL negative MPNs are distinguished by the presence of a JAK2, CALR, or MPL1 mutation. ET, PV, and PMF patients all have the same genetic markers and can only be separated by clinical criteria.

For many years, the diagnosis of BCR-ABL-negative MPNs was mostly based on clinical signs, cytomorphology, and histomorphology. The World Health Organization [2001][1] includes cytogenetic features both directly and indirectly in their categorization scheme.[2] Molecular markers are significant in the diagnosis of MPN. The presence of a chromosomal rearrangement at t[9;22][q34;q11], known as the Philadelphia [Ph] chromosome, distinguishes CML from the Ph-negative MPN, ET, PV, and PMF.

Somatic mutations, blood counts, and bone marrow biopsies identify these three MPN syndromes. PV is diagnosed by a somatic mutation in JAK2, high red cell mass, and bone marrow hypercellularity with trilineage expansion. ET is identified by increased platelets and megakaryocyte proliferation in the bone marrow. The presence of megakaryocytic proliferation and atypia, as well as reticulin and/or collagen fibrosis, is required for the diagnosis of PMF. The prefibrotic stage and the overt stage of myelofibrosis differ in terms of fibrosis severity, megakaryocyte shape, and the presence of leukoerythroblastosis.[3]

In addition to clinical indicators, the presence of a somatic mutation in JAK2, MPL, or CALR is required for the diagnosis of ET and PMF. Even in

individuals with "triple-negative" MPN, when the causal gene mutation is unclear, hyperactive JAK/STAT signalling appears to be the common denominator. Changes in epigenetic regulators, transcription factors, and signalling components alter disease progression and can contribute to illness onset and progression. Because of JAK2's essential function in MPN, tiny molecule inhibitors have been developed that are already in clinical use and are active in virtually all MPN patients. The precise involvement of JAK2, MPL, and CALR mutations in the pathophysiology, phenotype, and consequences of the three MPN subtypes remain unknown. None of the JAK2-V617F, MPL, or CALR mutations are associated with a specific MPN subtype.[3]

The WHO considers the combination of clinical, morphological, and molecular genetic traits to be the most appropriate approach to describe disease entities such as MPNs.[3,4] The following tests are necessary to detect MPNs:

1. Peripheral blood film examination, which can indicate hyperleucocytosis with or without blasts, anaemia, thrombocytosis, or pancytopenic smears, and a leucoerythroblastic image.
2. Serological testing, including serum erythropoitine levels, which will be lower in polycythemia vera and higher in primary myelofibrosis.
3. Bone marrow aspiration and biopsy will reveal age-adjusted hypercellularity in the majority of MPNs with hyperplasia in one or more series. Fibrosis of varying severity might be noticed.
4. Molecular cytogenetics for diagnostic confirmation

Clinical manifestations include: Weight loss, nocturnal sweats, and fever are common presenting complaints. Patients with polycythemia vera and essential thrombocythemia may have vascular anomalies. Organomegaly manifests as splenomegaly and hepatomegaly as a result of excess blood cell sequestration and the proliferation of aberrant haematopoietic progenitor cells. Erythrocytosis indicates PV, thrombocytosis indicates ET, and a leukoerythroblastic smear is one of the hallmarks of PMF. In the

presence of palpable splenomegaly, anaemia, pruritus, anomalous thrombosis such as portal or hepatic vein thrombosis, bone marrow fibrosis, and extramedullary hematopoiesis, myeloproliferative neoplasm should be considered.

Chronic myeloid leukaemia [CML] is a clonal myeloproliferative neoplasm caused by neoplastic transformation of pluripotent stem cells that is linked to the BCR-ABL 1 fusion gene on the Philadelphia chromosome. CML is distinguished by the uncontrolled production of mature granulocytes, primarily neutrophils but also eosinophils and basophils. CML offers a three-tiered course:

• Massive leucocytosis in its chronic phase [CP].

• Accelerated phase [AP] with rapid increase of blast cells and clinical deterioration.

• Blast crisis, with blasts in bone marrow exceeding 20%, resulting in rapid clinical deterioration.

Polycythemia vera [PV] is a panmyelosis-like stem cell disease. The most noticeable aspect is an increased absolute red blood cell mass as a result of unregulated red blood cell production. This is accompanied by increased production of white blood cells [myeloid] and platelets [megakaryocytic], which is caused by an aberrant clone of hematopoietic stem cells with greater sensitivity to various growth stimuli for maturation. The trephine biopsy and bone marrow aspiration reveal enhanced cellularity with trilineage cell proliferation.

Myelofibrosis frequently hinders bone marrow cytology in PMF, and peripheral blood reveals poikilocytosis and leuko-erythroblastosis. These features, however, are not specific and can be found in secondary myelofibrosis following other CMPD[2]. ET is distinguished by megakaryocytic lineage proliferation, as seen by clusters of larger but mature megakaryocytes in normocellular bone marrow. There are clusters of enlarged mature megakaryocytes near to the sinusoids on bone marrow trephine biopsy, but erythropoiesis and granulopoiesis are

normal. Chronic neutrophilic leukaemia [CNL] is a rare myeloproliferative neoplasm distinguished by persistent peripheral blood neutrophilia, bone marrow hypercellularity due to neutrophilic granulocyte growth, and hepatosplenomegaly. There is no BCR-ABL1 fusion gene. In roughly 90% of cases, cytogenetic investigations are normal. CSF3R activating mutations have recently been identified in CNL patients. Chronic eosinophilic leukaemia, not otherwise defined [CEL NOS] is characterised by clonal proliferation of eosinophil precursors, leading to persistently elevated eosinophil counts in the peripheral blood, bone marrow, and peripheral tissues. Organ damage occurs as a result of leukemic infiltration or release of cytokines, enzymes, or other proteins by eosinophils. Unclassified myeloproliferative neoplasms are identified when clinical haematological and molecular factors favour MPNs but do not fulfil any particular criteria of entities included in MPNs.[2]

These illnesses are progressive, with patients progressing from one to the next over time. In the final stage, myelofibrosis may cause marrow failure or transition to acute blast phase. Fibrosis can also be detected as a result of growth factors produced by platelet and megakaryocytes.

AIMS AND OBJECTIVES

To correlate the genetic profile of patients with myeloproliferative neoplasm with hemogram, bone marrow aspiration and biopsy findings.

REVIEW OF LITERATURE

The myeloproliferative neoplasms [MPNs], previously termed the myeloproliferative disorders, are characterized by the clonal proliferation of one or more hematopoietic cell lineages [ie. granulocytic, erythroid and megakaryocytic]. The proliferation of a clone of neoplastic cells is derived from a mutated hemopoietic stem cell. Though the proliferation capacity is not controlled resulting in increased number of cells, they still undergo complete range of maturation and function normally. There is a variable degree of myeloid, erythroid and megakaryocytic hyperplasia with predominance of one series resulting in overproduction of white cells in chronic myeloid leukemia [CML]; red cells in polycythemia vera[PV]; and platelets in essential thrombocythemla[ET]. Bone marrow was first obtained from living patients for diagnostic purposes [for the diagnosis of leishmaniasis] during the first decade of the twentieth century; this was reported from Italy by M. Pianese and from Germany by P. Wolff, following puncture of the femur and tibia respectively.[5]

In October 1845, John Hughes Bennett, an English pathologist at the Royal Infirmary in Edinburgh, Scotland, published the first report of CML entitled ‘Case of hypertrophy of the spleen and liver in which death took place from suppuration of the blood.[6]

In 1951, William Dameshek explained the concept of ‘myeloproliferative disorders [MPDs]’ by putting together chronic myelogenous leukemia [CML], polycythemia vera[PV], essential thrombocythemia[ET], primary myelofibrosis [PMF] and erythroleukemia.He put forward the idea of these entities representing a chain of linked syndromes and the proliferative activity is perhaps due “to a hitherto undiscovered stimulus”[7]

Gustav Heuck,German surgeon was the first to describe PMF, in 1879, under the title of ‘Two cases of leukemia with peculiar blood and bone marrow findings’.Heuck described two young patients with massive splenomegaly, circulating nucleated red blood cells, and increased

number of morphologically abnormal leukocytes. He recognized that the findings in his two patients differed from CML because of the presence of marrow fibrosis and extensive extramedullary hematopoiesis [EMH].[8]

Louis Henri Vaquez, a French physician, was the first to describe PV in 1892 in a 40-year-old man with chronic ‘cyanosis’ distended veins, vertigo, dyspnea, palpitations, hepatosplenomegaly and marked erythrocytosis.[9]

Essential thrombocythemia formally described as ‘hemorrhagic thrombocythemia’ in 1934 by Emil Epstein and Alfred Goedel, both Austrian pathologists in patient with extreme thrombocytosis, slight erythrocytosis and recurrent mucocutaneous bleeding, absence of prominent panmyelosis.[10]

In 1960, Nowell and Hungerford discovered the Philadelphia [Ph] chromosome in CML.In 2005, D Gary Gilliland, William Vainchenker, Radek Skoda and Anthony Green discovered that the majority of patients with classic BCR–ABL-negative MPDs carried a JAK2 gain-of-function mutation [7]

Pathogenesis

To maintain the number of blood cells or to adjust them under physiological stress, internal and external regulators control hematopoiesis by balancing proliferation, differentiation and apoptosis. External regulators, like cytokines and growth factors, bind and induce signaling through their cognate cell surface receptors. Ligand binding of receptors is integrated by signaling cascades such as the JAK/STAT, RAS/MAPK and the PI3K/mTOR pathway. These cascades control internal regulators like transcription factors or miRNAs, which regulate hematopoiesis by adjusting expression of target genes at RNA level.

Most myeloproliferative neoplasms are associated with clonal abnormalities either involving genes that encode cytoplasmic or receptor protein tyrosine kinases causing constitutive activation of oncogenic

signaling pathways or activating regulators of these pathways resulting in similar biological consequences.

The mutational abnormalities include translocations, insertions, deletions, and point mutations of genes resulting in abnormal, constitutively activated protein tyrosine kinases that activate signal transduction pathways, leading to abnormal proliferation. In some cases, these genetic abnormalities are associated with clinical, laboratory, and morphological findings, which enables their use as major criteria for classification. Presence of genetic abnormalities provides proof that the myeloid proliferation is neoplastic rather than reactive.

In CML the Philadelphia chromosome is the sole genetic requirement for diagnosis, the Ph-negative MPN phenotypes share mutations in the genes JAK2, MPL and CALR.

The JAK2-V617F mutation, is a single nucleotide point mutation in exon 14 of the JAK2 gene and is found in ET, PV and PMF patients.[11]The prevalence of varies among the Ph-negative MPN is depending on the disease phenotype: nearly all PV patients [90%] carry this mutation, whereas only 50-60% of the patients diagnosed with ET or PMF are JAK2-V617Fpositive.[12] JAK2 exon 12 mutations are exclusively found in PV patients.[13]

Another gene recurrently mutated in Ph-negative MPN is the thrombopoietin receptor MPL. MPL mutations changing the codon of the tryptophan at position 515, are found in about 5% of ET and PMF patients, but are absent in PV patients.[14]

The third gene recurrently mutated in ET and PMF is Calreticulin[CALR]. CALRis affected by insertion or deletion mutations, which lead to a frame shift in exon 9. *CALR* mutations are absent in PV patients [15]

Hyperactive JAK/STAT signaling is the common denominator of MPNs, even in which are "triple-negative" MPN and the driver gene mutation is still unknown. Mutations in epigenetic regulators, transcription factors, and

signaling components modify the course of the disease and can contribute to disease initiation and/or progression.[16]

Myeloproliferative neoplasms

The correct diagnosis and classification of MPN requires a structured approach in which clinical features and peripheral blood, bone marrow cytological, histological, cytogenetic and molecular characteristics are all assessed.[3,4] Morphology remains the central distinguishing feature in the 2016 WHO system for classification of tumors of the hematopoietic and lymphoid tissues, although mutation screening is increasingly being utilized for confirmation of morphologic diagnosis and, at times, for directing the diagnostic process.[3]

The 2016 WHO category of MPNs includes the BCR-ABL positive, CML and BCR-ABL negative entities that are positive for JAK2/CALR/MPL mutation related MPNs [i.e., PV, ET, and PMF], as well as other clinicopathologic entities: chronic neutrophilic leukemia [CNL], chronic eosinophilic leukemia, not otherwise specified [CELNOS] and MPN, unclassifiable [MPN-U].

The entities differ from each other in predominate type of differentiation that the neoplastic cell undergoes i.e myeloid in chronic myeloid leukemia [CML], erythroid in polycythemia vera [PV], megakaryocytes in essential thrombocytosis [ET], excessive fibrosis along with extramedullary hematopoiesis in primary myelofibrosis [PMF].

Numerous variants, transitional and intermediate forms has been recognized, transformation to blast crisis, to secondary myelofibrosis and to other hematopoietic malignancies.

WHO classification

The first international consensus classification of hematologic tumors by WHO was published in 2001. It is based on the principles initially defined in the Revised European-American classification of lymphoid

neoplasms [REAL], proposed by the International Lymphoma Study Group. This was followed by 4th edition published in 2008, update of the 3rd edition.[17]

i. The term myeloproliferative disease was replaced by myeloproliferative neoplasm.
ii. The entity CEL/HES [3rd edition] was reorganized as CEL, NOS as part of MPN and myeloid and lymphoid neoplasm with eosinophilia and abnormalities of PDGFRA, PDGFRB and FGFR1.
iii. The diagnostic algorithms for PV, ET and PMF were changed, including mutational status of JAK2 gene and other correlate genes. Bone marrow histological findings, particularly for megakaryocytes, were strengthened as diagnostic criteria.
iv. The platelet count threshold for ET was reduced from 600,000/mm3 to 450,000/mm3.

2008 WHO classification of myeloproliferative neoplasm:

1. Chronic myelogenous leukemia, BCR-ABL positive [CML]
2. Polycythemia vera [PV]
3. Essential thrombocythemia [ET]
4. Primary myelofibrosis [PMF]
5. Chronic neutrophilic leukemia [CNL]
6. Chronic eosinophilic leukemia, not otherwise specified [CEL,NOS]
7. Mast cell disease
8. MPN, unclassified

The 2016 revision of 2008 World Health Organization [WHO] classification of tumours of haematopoietic and lymphoid.[18]

i. Discoveries of new mutations and improved understanding of the morphologic features of some entities have impacted the diagnostic criteria for the disease entities
ii. Term chronic myelogenous leukaemia, BCR-ABL 1positive has been changed to chronic myeloid leukaemia, BCR-ABL 1- positive.

iii. Mastocytosis, however, is no longer considered a subgroup of the MPNs due to its unique clinical and pathologic features, ranging from indolent cutaneous disease to aggressive systemic disease

iv. The criteria for AP in the revised WHO classification include hematologic, morphologic, and cytogenetic parameters which are supplemented by additional parameters usually attributed to genetic evolution,5 and manifested by evidence of resistance to TKIs

v. For BCR-ABL negative MPNS. The criteria for AP in the revised WHO classification include hematologic, morphologic, and cytogenetic parameters which are supplemented by additional parameters usually attributed to genetic evolution,5 and manifested by evidence of resistance to TKIs

vi. The CSF3R mutation is strongly associated with chronic neutrophilic leukemia

vii. Improved characterization and standardization of morphological features aiding in histological pattern recognitionand differentiation of disease groups has increased the reliability and reproducibility of diagnosis

2016 classification of MPNs:

1. Chronic myeloid leukaemia, BCR-ABL1- positive
2. Polycythaemiavera
3. Primary myelofibrosis
4. Essential thrombocythaemia
5. Chronic eosinophilic leukaemia,
6. Myeloproliferative neoplasm, unclassifiable

Laboratory diagnosis

The combination of clinical, morphological, and molecular genetic features is thought by the WHO as the most suitable attempt to define disease entities such as MPNs.[3,4] Following the updated 2008 WHO classification[4], a number of clinical–pathological studies conducted by

different groups have validated these diagnostic guidelines including the importance of morphologicalfeatures.[19]

However, a balanced and evidence based discussion concerning these diagnostic criteria persists.[20] In particular, it has been postulated that ET, PV, and PMF cannot be strictly discriminated by BM morphology as postulated by the WHO, owing to their mimicry to transform to each other.[21] Differential diagnosis of MPNs is made based on peripheral blood smears, bone marrow aspirates and trephine biopsies.[2]

Cytomorphology requires 3 ml bone marrow and 2 ml peripheral blood anticoagulated with ethylene diaminetetraacetic acid [EDTA], thecytomorphology is hampered by heparine.

Cytogenetics requires 5–10 ml heparinized bone marrow and 10–20 ml heparinized peripheral blood.The cultivation of metaphases is inhibited by EDTA which induces apoptosis of cells.[2]

Molecular genetic methods can be performed either on EDTA or heparinized samples.

Trephine biopsies are done for histomorphology and immunohistochemistry. It also allows cytomorphological evaluation in case of a dry tap.

Most MPNs characterized by varying degrees of age adjusted hypercellularity of the bone marrow, with effective haematopoietic maturation and increased numbers of granulocytes, red blood cells and platelets in the peripheral blood. Splenomegaly and Hepatomegaly due to sequestration of excess blood cells and proliferation of abnormal haematopoietic progenitor cells. PMF requires bone marrow histology for diagnosis, as bone marrow aspirates are a dry tap in most cases. Histology shows variable reticulin or collagen fibrosis, osteosclerosis, and decreased cellularity. Sinuses are dilated with intraluminal hematopoiesis and erythrocyte extravasates. Megakaryopoiesis is increased with signs of dysplasia.

Cytogenetics and molecular testing

The methods used to detect the genetic changes in hematologic malignancies include:

[a] Conventional cytogenetics [karyotyping on cells derived from direct preparations or short-term cultures using banding analysis; G-banding]

[b] Molecular cytogenetics, e.g. fluorescence in situ hybridization [FISH], multicolor FISH, and spectral karyotyping [SKY]

[c] Molecular techniques to analyze DNA, RNA, or proteins directly, e.g. the polymerase chain reaction [PCR], reverse transcriptase PCR [RT-PCR], quantitative real-time RT-PCR [RQ-PCR; qRTPCR], Southern blotting, and microarray analysis.

Conventional cytogenetics

Classical karyotyping of chromosomes remains the most comprehensive method for assessing chromosome abnormalities, especially numeric and structural chromosome aberrations in conventional cytogenetics.

Karyotyping- A karyotype is the complete set of chromosomes in a cell. Karyotyping is the direct observation of metaphase chromosome structure by arranging metaphase chromosomes according to size. Karyotyping requires collecting living cells and growing them in culture in the laboratory for 48–72 hours. Cell division is stimulated by addition of a mitogen. Dividing cells are then arrested in metaphase with microtubule inhibitor. The chromosomes in dividing cells that arrest in metaphase will yield a chromosome spread when the cell nuclei are disrupted with hypotonic saline. The 23 pairs of chromosomes can then be assembled into an organized display, or karyotype according to their size and centromere placemen. The detection of any abnormality in at least two cells is required to confirm clonality.[22]

A significant subset of hematopoietic malignancies shows lack of chromosomal rearrangements in conventional cytogenetic studies and only molecular tests are able to visualize the underlying genetic defects.

Molecular cytogenetics

Fluorescence in situ hybridization

FISH - test probes are used against target DNA in the nucleus of interphase or metaphase chromosomes. In myeloproliferative neoplasm the most common chromosomal abnormalities targeted by FISH is t[9;22]BCR/ABLin CML.[22]

Methodology– The FISH protocol includes:

-denaturation, in this the probe and target DNA are denatured by incubating at high temperature.

-hybridization, the probe is hybridized tothe chromosomal target.

-washing to remove theunbound probe.

-analysis under the fluorescence microscope.

DNA is analyzed in the cell or on the chromosome. It is not extracted and run in a gel.[22]

Indirect and direct FISH

1. In the indirect FISH method, biotin or digoxigenin labeled nucleotides are visualized by fluorescein isothiocyanate[FITC]-conjugated antidigoxigenin antibodies.
2. In direct method the probes are already labeled with fluorochromes. For detection of translocation, two probes are labeled with different fluorochromes. In a normal cell four signals [two of each color] ae detected, while in a cell with translocation two adjacent signals leading to a different colored fluorescence signal is detected.[22]

Two types of probes are used to detect translocations:

1. single or dual fusion probes
2. break-apart probes.

A dual-fusion probe consists of a pair of probes labeled with two different colors, green and red directed against translocation breakpoint regions in the two different genes involved in a reciprocal translocation. In a normal cell there are two green and two red signals corresponding to two separate loci that are not in close proximity i.e there is no translocation. In cells with translocation between the targeted loci, there will be one green and one red signal and one yellow signal indicating the fusion between two loci [yellow fluorescence being the result of overlap between the green and red signals].[22]

FISH probes:

[a] Centromere-specific probes - can detect specific chromosomes, and therefore are useful to demonstrate numeric chromosomal aberrations in metaphase or interphase nuclei

[b] Whole chromosome [‘painting’] probes - consist of numerous overlapping probes that recognize and bind to specific nonrepetitive DNA sequences along the entire length of targeted chromosomes. These are used to identify rearranged chromosomes of unidentified origin or translocations that are otherwise not evident or difficult to interpret with routine banding cytogenetics.

[c] Single-copy [locus-specific] genomic probes - used to detect the presence of amplification i.eincreased number of copies of a specific locus or to detect the presence of rearrangement

[d] Spectral karyotyping [SKY; multiplex metaphase FISH; multicolor FISH] - is a novel 24-color, multichromosomal painting technique, that allows for the simultaneous visualization of all chromosomes of a metaphase in a single hybridization step. It allows simultaneous identification of the 22 autosomes and the 2 sex chromosomes.

Molecular techniques to analysis DNA, RNA and protein

Polymerase chain reaction

It targets a segment of DNA or RNA in reverse-transcriptase PCR and produces multiple copies.

Methodology- denaturation of double-stranded DNA into single-stranded DNA

- Hybridization [annealing] of oligonucleotide primers to both ends of a targetsequence.
- Synthesis of new DNA strands achieved by addition of four nucleotide bases and a Taq polymerase using oligonucleotide primers
- PCR reaction usually involves 30–40 cycles, denaturation is doneat high temperature [95°C]

Reverse transcriptase -PCR- amplifies RNA and consists of two major steps:

1. An enzyme, reverse transcriptase converts RNA into the single stranded complementary DNA.
2. Single stranded complementary DNA serves as template for conventional PCR.

The original RNA is degraded by RNase. It used in the diagnosis of genetic diseases, since it makes it easier to detect the presence of specific aberrations by directly detecting the product mRNA.

REAL time polymerase chain reaction –

- **Qualitative PCR**
- **Quantitative PCR**

Qualitative PCR fails to detect disease-specific changes in certain subsets of patients, especially in patients monitored for MRD. This is often associated with a poor quality of RNA.

Quantitative PCR [qRT-PCR] detects the earliest stages of molecular relapse. This technique does simultaneous amplification and detection of PCR products in a single reaction tube.

The reaction is monitored in real time after each cycle of amplification by computer software permitting both detection and quantification of PCR products. After each amplification cycle, a dye is released and its amount is measured in real time for quantitation of DNA in the exponential phase. The results are presented in the form of a graph which plots the dye corresponding to the amount of the starting template versus the number of PCR cycles. The quantitation is based on the number of cycles required to reach a designated threshold [the lower the number of cycles to reach the threshold the higher the expression of the mRNA or DNA copy number]. This is using the fluorescence-based quenching methods. The most commonly used systems for qRT-PCR include theTaqMan system, Molecular beacons, Scorpions, and SYBR green.

Molecular technology, especially real-time quantitative RT-PCR [qRT-PCR], plays an important role in monitoring patients after treatment, best documented in CML patients on imatinib.[23,24]

Gel electrophoresis

Gel electrophoresis [most often using polyacrylamide gels; PAGE] is used to separate DNA products based on their size. DNA is then visualized by chemical stains unlabeled probes orfluorochrome-labeled probes. Capillary electrophoresis employs thin glass tubes in which DNA products are separated and then visualized by either emitted fluorescence or under UV light absorbance.[22]

Next generation sequencing

It performs sequencing of millions of small fragments of DNA in parallel. NGS can be used to sequence entire genomes or specific areas of

interest. The technology is used to determine the order of nucleotides in entire genomes or targeted regions of DNA or RNA.

Spatial separation- The individual DNA molecules are isolated from each other and are amplified in situ using a limited number of PCR cycles. Local amplification- doneto generate sufficient signal to ensure detection and accuracy.Parallel sequencing-The amplified DNA molecules are simultaneously sequenced by adding polymerases yielding a “read” corresponding to its sequence.[22]

Common genetic mutations

BCR-ABL

The t[9;22] translocation occurs in CML.Ph chromosome, a shortened chromosome 22 results from a reciprocal translocation between the *BCR* gene on chromosome 22 and the ABLgene on chromosome 9.

The t[9;22] or its molecular equivalent can be detected in virtually all cases of CML by PCR, FISH, conventional cytogenetics or Southern blot. The fusion gene BCR/ABL, encodes a chimeric protein with tyrosine kinase activity.

Depending on the position of the *BCR* breakpoint, fusion genes are generated that encode 190 kD[minor breakpoint cluster region], 210 kD[major breakpoint cluster region], or 230 kD forms of the bcr-abl tyrosine kinase. Each fusion gene encodesthe same portion of the ABL tyrosine kinase but differs in the length of BCR sequence retained at the N terminal. Nearly all patients with typical chronic-phase CML express a 210-kd BCR–ABL protein.[22]

CML expressing p190 is relatively rare. Patients with p190 have a worse prognosis than those with p210.[25] A quantitative molecular assay for bcr-abl RNA [qRT-PCR] can be used to monitor patients with CML after treatment and progression of CML, including accelerated phase [AP] or BP [blast crisis], which is often associated with chromosomal evolution, i.e. the appearance of chromosomal abnormalities in addition to the Ph

chromosome. Which may include additional copies of the Ph chromosome, isochromosome17, a gain of chromosomes 8 or 19.[26]

BCR-ABL has several substrates and impacts on key signalling pathways resulting in the CML phenotype. The net result is deregulated cellular proliferation and development of growth factor independence, decreased adherence of the leukaemic cells to the bone marrow stroma, and a reduced apoptotic response to mutagenic stimulus.[27]

JAK2

JAK2 is a non-receptor tyrosine kinase, which, upon ligand binding to specific cytokine receptors, is phosphorylated and activated, leading to regulation of gene expression involved in cell proliferation and survival. The JAK2V617F mutation is a G to T somatic mutation at nucleotide 1849 in exon 14, resulting in the substitution of valine to phenylalanine at codon 617, which triggers constitutive activation of downstream signaling and uncontrolled cell growth.[28]

The JAK2-V617F mutation is a single nucleotide point mutation in exon 14 of the JAK2 gene and is found in ET, PV and PMF patients. The Janus family of cytosolic tyrosine kinases [JAK] plays an essential role in development and normal hematopoiesis. JAK2, encoded on chromosome 9p24, plays a central role in nonprotein tyrosine kinase receptor signaling pathways.[22] Somatic point mutations of the tyrosine kinase JAK2 [JAK2V617F] have been reported in ≥95% of PV but they can be also seen in ET and PMF. Quantitative PCR methods [qPCR] are preferred over qualitative ones because of greater reproducibility and sensitivity and because of the need of quantifying the mutated clone in MPN patients. The presence of the JAK2 mutation and identification of an abnormal karyotype are very useful in the management of patients with erythrocytosis, since it helps to exclude secondary etiology.[28,29,30]

MPL

The myeloproliferative leukemia virus oncogene [MPL] is located on chromosome 1p34 and encodes for the thrombopoietin[TPO] receptor, thus assuming a crucial role in the regulation of megakaryocyte growth and survival. In 2006, a somatic activating mutation in exon 10 of this gene, MPLW515L, was described in JAK2V617F-negative ET/PMF .This mutation is characterized by a G to T transition at nucleotide 1544, resulting in a tryptophan to leucine substitution at codon 515 of the transmembrane region of MPL, inducing constitutive activation of the TPO receptor in a cytokine-independent fashion.[28,29]

The thrombopoietin receptor TpoR known as myeloproliferative leukemia protein MPL, is a receptor, which specifically binds the cytokine thrombopoietin[TPO] and signals through the AK/STAT pathway. MPL is expressed by hematopoietic stem and progenitor cells megakaryocytes and platelets. Upon binding TPO, MPL activates JAK/STAT, MAPK and PI3K-AKT1-mTOR pathways resulting in survival and proliferation.[31]

CALR

CALR is a multi-functional Ca2+ binding protein with chaperone activity mainly localized in the endoplasmic reticulum. The localization and retention of *CALR* are defined by the N-terminal signal sequence and the C-terminal ER-retention sequence. It is involved in numerous intracellular [cytoplasm and nucleus], cell surface, and extracellular functions such as protein quality control, calcium metabolism, immune response, phagocytosis, cell adhesion and others. CALR mutations were phenotypic drivers in the pathogenesis of MPNs.[29]

It is a protein is located in the endoplasmic reticulum [ER], The specific location and length of the deletions and insertions vary within the exon 9 of CALRmutations result in an ET, there is amplification of the megakaryocyte lineage and elevated platelet counts. Studies have shown

the disease frequently progress to myelofibrosis phenotype including features of anemia, splenomegaly and BM hypocellularity.[32]

Calreticulin[CALR] mutations were reported for the first time at the end of 2013.[33]

These mutations represent the second most common genetic abnormality in MPNs after JAK2V617F, even though they are absent in PV patients. On the other hand, CALR mutations partially addressed the molecular gap in JAK2/MPL-unmutated ET and PMF patients, accounting for 20–25% of the overall somatic mutations. The remaining cases [i.e., negative for JAK2, CALR, and MPL] are termed “triple-negative,” representing the 5–10% of all BCR-ABL1-negative MPN patients.[29]

Given their high frequency in MPNs, screening for CALR mutations is recommended in all JAK2V617F-negative patients with suspected ET or PMF

Chronic myeloid leukemia [CML]

Now designated chronic myeloid leukemia, BCRABL1 positive, previously also known as chronic granulocytic leukemia and chronic myelogenous leukemia.

Pathogenesis- Chronic myeloid leukemia [CML] have an acquired chromosomal abnormality in the leukemic clone consisting of a reciprocal translocation between the long arms of chromosomes 9 and 22, t[9;22][q34.1;q11.2]. The abnormal chromosome 22 [22q–] is known as the Philadelphia [Ph] chromosome. Resulting into a hybridgene formed on 22q–, by fusion of part of the ABL1[Abelson] oncogene from the long arm of chromosome 9 with part of a chromosome 22 gene known as BCR[breakpoint cluster region].[34]

This fusion gene encodes an abnormal tyrosine kinase. Patients whose cells lack the t[9;22] and the resultant Ph chromosome, but in whom the disease is identical to Ph-positive disease in all other respects, have a BCR-ABL1fusion gene demonstrable by molecular techniques.[1] Fusion

protein BCR–ABL is essential for initiation, maintenance and progression of CML, yet the transformation of CML from chronic phase to blast phase requires additional genetic and/or epigenetic abnormalities.

The time taken from initial genetic event to fully established chronic-phase CML is not known, but there is reason to believe that the proliferative advantage of CML over normal cells is limited. Together with the normal differentiation capacity and function of CML blood cells, the Ph-positive hematopoiesis is not much different from normal hematopoiesis until the disease accelerates.[34]

Thus, BCR-ABL is likely to hijack pathways that normally increase blood cell output in response to physiologic stimuli rather than to interrupt or replace them with pathways that are not normally used in hematopoietic cells. Importantly, BAR-ABL is capable of activating survival pathways along with proliferative stimuli.[34]

Morphologically granulocytes are major proliferative component. The diagnosis requires detection of the Phchromosome and/or BCR-ABL *1* in the appropriate clinical and laboratory settings

The chronic myeloid leukemia has three phases:

1. Chronic phase [CP]
2. Accelerated phase [AP]
3. Blast crisis

In Chronic phase- the leukemia cells are minimallyinvasive and mostly confined to the blood, bone marrow, spleen, and liver. In BP, the blasts can infiltrate any extramedullary site, with a predilection for spleen, liver, lymph nodes, skin, and soft tissue.

Clinical presentation- Symptoms include anemia, abdominal fullness, splenic discomfort or pain due to splenomegaly, fatigue, malaise, weight loss, night sweats, unexplained fever. Atypical presentations include marked thrombocytosis without leukocytosis. However, because of the insidiousonset of the disease, many patients have only minorsymptoms at

the time of diagnosis. The disease is often diagnosed incidentally from a blood count in an asymptomatic patient.[35]

Physical examination- usually reveals splenomegaly although not massive until the WBC exceeds 100 × 10^9/L. hepatomegaly is also common.

Peripheral blood film examination–TheWBC countis elevated, usually between 20 and500 × 10^9/L.[36]The predominant cell types being neutrophils and myelocytes along with immature granulocytic cells. The blast cells and promyelocytes usually being less than 10–15% of cells.[37]Basophils are also invariably increased and the absolute eosinophil count is increased in the great majority of patients.

A normocytic normochromic anemia is seen. The platelet count is usually normal or elevated.

Bone marrow aspiration and biopsy examination- shows a hypercellular marrow with normal range of maturation of cells. There is increase in granulocytes and their precursors along with increase in megakaryocytes.[38] The myeloid to erythroid ratio is increased. Precursors of neutrophils, eosinophils andbasophils are all increased. Cellular maturation is normal.[39] Erythropoiesis is reduced but morphologically normal. The average size and lobation of megakaryocytes is reduced,hyposegmented nuclei referred to as 'dwarf' megakaryocytes are seen.[40] Moderate to marked reticulin fibrosis, correlating with increased numbersof megakaryocytes is noted and may be associated with an enlarged spleen. As a consequence of the increased cell turnover, macrophages and various storage cells are often prominent.[41,42]

Macrophages with crystalloid inclusions-pseudo-Gaucher cells can be seen.[42]

Accelerated phase- the bone marrowshow increase in blast cells or the appearance of dysplastic features, such as micromegakaryocytes. Bone marrow aspiration may become difficult or impossible because of increase in bone marrow fibrosis.

Differential diagnosis- Leukemoid reaction can be differentiated from CML by presence of leukocytosis consisting predominantly of mature neutrophils and myelocyte and metamyelocytes with raised LAP score as compared to low LAP score in CML. The biopsy sections will reveal the abrupt transition from myeloblasts to mature granulocytes. Polymorphism of megakaryocytes will be absent.[35]

The 2016 revision of the World Health Organization [WHO] classification of tumors of haematopoietic and lymphoid tissues criteria for diagnosis of three phases of CML.

CML Chronic phase-

1. Hypercellular marrow with myeloid hyperplasia and expansion of myelocyte stage.
2. Blasts <5% of marrow cells

CML Accelerated phase-

defining criteria is presence of >1 of the following hematological/cytogenetic criteria or provisional criteria concerning response to tyrosine kinase inhibitor [TKI] therapy

Hematological/cytogenetic criteria

1. Persistent or increasing high TLC count [> 10 x 10^9/L], unresponsive to therapy
2. Persistent or increasing splenomegaly, unresponsive to therapy
3. Persistent thrombocytosis [> 1000 x 10^9/L], unresponsive to therapy
4. Persistent thrombocytopenia [< 100 x 10^9/L], unrelated to therapy
5. >20% basophils in the peripheral blood
6. 10-19% blasts in the peripheral blood and/or bone marrow

Additional clonal chromosomal abnormalities in Philadelphia [Ph] chromosome-positive [Ph+] cells at diagnosis.

Provisional response-to-TKI criteria

1. Hematological resistance [or failure to achieve a complete hematological response] to the first TKI

2. Any hematological, cytogenetic, or molecular indications of resistance to two sequential TKIs
3. Occurrence of two or more mutations in the BCR-ABL1fusion gene during TKI therapy

CML Blast phase-

The criteria for BP include >20% blasts in the blood or bone marrow or the presence of an extramedullary proliferation of blasts.[43] Blast transformation may involve part or all of a trephine biopsy specimen. Areas of involvement contain sheets of blast cells, which usually have a single prominent nucleolus and often show considerable pleomorphism.[44,45,46]

Immunohistochemistry is of limited value during the chronic phase of the disease but can be useful in the accelerated phase and in blast transformation[46]. Increased numbers of CD34-positive cells are observed in the accelerated phase and transformation[47] and generally correlate with an increase of blast cells. Immunohistochemistry can be useful to distinguish between myeloid and lymphoid transformation

Cytogenetics- Chronic myeloid leukemia is typically associated with t[9;22][q34.1;q11.2] and with the formation of a BCR-ABL1 fusion gene. The diagnosis of CML cannot be sustained if BCRABL1 is lacking. The BCR-ABL1 fusion gene usually encodes a 210 kD protein with tyrosine kinaseactivity, which has an important role in the pathogenesis of the disease.[22] The diagnosis of CML can be confirmed by conventional cytogenetic analysis, fluorescencein situ hybridization [FISH] or a reverse transcriptase polymerase chain reaction [RT-PCR].[34]

Polycythemia vera [PV]

Polycythemia vera [PV], previously known as polycythemia rubra vera or primary proliferative polycythemia, is an MPN in which there is excessive

production of erythrocytes by the marrow resulting in increase in the circulating red cell mass and the venous haematocrit. Frequently there is an increase in cells of other hemopoietic cell lineages, both in the marrow and in the peripheral blood, reflecting the origin of the neoplastic clone from a multipotent myeloid stem cell.[35]

Clinical presenting features- are related to hyperviscosity of the blood and to the arterial or venous thromboses that occur; these include headache, a feeling of fullness of the head, dizziness, tinnitus, dyspnoea, visual disturbance, Raynaud's phenomenon, erythromelalgia, claudication and gangrene. Pruritus can occur, probably consequent on histamine secretion by basophils. Up to 70% of cases have been found to have splenomegaly and 40% hepatomegaly. [chapter 5, bone maroow pathology, Barbara bain. Fifith edition].[39] Physical findings include plethora, palpable splenomegaly and hepatomegaly.

Differential diagnosis- Polycythemia vera must be distinguished from secondary polycythemia, which is a consequence of either chronic generalized tissue hypoxia- due to high altitude, chronic hypoxic pulmonary disease or cyanotic congenital heart disease, inappropriate erythropoietin production resulting from chronic renal hypoxia or ectopicproduction of erythropoietin by a renal or other tumor or intake of androgenic steroids.Polycythemia vera and secondary polycythemia must also be distinguished from pseudo- or relative polycythemia, which is a consequence of a decreased plasma volume rather than an increased red cell mass.Some patients in whom no underlying cause of secondary polycythemia can be found do not have any clinical, hematological or histopathological features that permit a diagnosis of PV. If such patients lack a detectable *JAK2* mutation [exon 12, exon 14 or other] the appropriate diagnosis is 'idiopathic erythrocytosis'.[35]

Polycythemia vera may enter a 'burnt out' or 'spent' phase in which there is initially a reduction of red cell production followed by the development of splenomegaly and bone marrow fibrosis; with progression and

development of all the clinical and pathological features usually associated with primary myelofibrosis. The designation ‘spent phase of polycythemia’ has been suggested for the phase of the disease when there is a stable hematocrit and marked splenomegaly with minimal fibrosis and the designation ‘transitional state polycythemia vera’ when there is persisting erythrocytosis with progressive myelofibrosis. The final stage of this progression is designated post-polycythemic myelofibrosis.[48]

Older age, leukocytosis, and abnormal karyotype have also been associated with a higher risk of leukemic transformation.[49]

On peripheral blood examination- there is elevation of the red cell count, Hb and hematocrit. As a consequence ofthe increased blood viscosity, the blood film shows crowding together of the red cells, an appearance described as a ‘packed film’. In some patients, iron stores have been exhausted and there is also microcytosis and hypochromia.[35]

On bone marrow examination- there is marked increase in erythroid precursors and often some increase of granulocytes, granulocyte precursors and megakaryocytes. Hypercellularity is especially note worthy in the subcortical marrow space, which is normally hypocellular. Megakaryocytes are pleomorphic, ranging from small forms with hypolobated nuclei to large forms with hyperlobated nuclei.[50]

Many cases show a mild increase in reticulin, with about 10% of patients showing a moderate or marked increase[51]

Reticulin fibrosis [grade 1 or more on a scale of 0–3] correlates with a lower frequency of thrombosis, a higher frequency of palpable splenomegaly and more frequent progression to post polycythemia myelofibrosis.[52]

Cytogenetics- A CALR mutation [a deletion or an insertion] is found in a third to a quarter of patients. Rarely JAK2V617F and a CALR mutation coexist.[52] Cases with a CALR mutation are distinct from JAK2-mutated cases with a lower thrombotic risk, no risk of transformation to PV and a lower risk of transformation to acute leukemia. In comparison with JAK2

mutation, CALR mutation is associated with younger age, male gender, higher platelet count, lower Hb and lower WBC.[52,53]

An MPL gain-of-function mutation in exon 10 of MPLhas been reported in 4–11% of patients and may coexist with JAK2V617F. MPL-mutated cases have a lower Hb than JAK2-mutated cases. Both CALRand MPLmutations lead to activation of MPL, the thrombopoietin receptor.[53]

The diagnosis of PV requires either all 3 major criteria or the first 2 major criteria plus the minor criterion.

Major criteria

1. Elevated hemoglobin concentration[> 16.5 g/dl in men;> 16.0 g/dl in women] or

Elevated hematocrit [>49% in men; >48% in women]

Increased red blood cell mass[> 25% above mean normal predicted value]

2. Bone marrow biopsy showing age-adjusted hypercellularity with trilineage growth [panmyelosis], including prominent erythroid, granulocytic, and megakaryocytic proliferation with pleomorphic, mature megakaryocytes [differences in size]

3. Presence of JAK2V617F or JAK2exon 12 mutation

Minor criterion

Subnormal serum erythropoietin level

Diagnostic criteria for post-polycythemia vera[PV] myelofibrosis

Required criteria

1. Documentation of a previous diagnosis of WHO-defined PV

2. Bone marrow fibrosis of: grade 2-3 on a 0-3 scale or grade 3-4 on a 0-4 scale

Additional criteria[2 are required]

1. Anemia [i.e. below the reference range given age, sex, and altitude considerations] or sustained loss of requirement of either phlebotomy [in

the absence of cytoreductive therapy] or cytoreductive treatment for erythrocytosis

2. Leukoerythroblastosis

3. Increasing splenomegaly, defined as either an increase in palpable splenomegaly of

> 5 cm from baseline [distance from the left costal margin] or the development of a newly palpable splenomegaly

4. Development of any 2 [or all 3] of the following constitutional symptoms: > 10% weight loss in 6 months, night sweats, unexplained fever[> 37.5 °C] Reticulin fibrosis [grade 1 or more on a scale of 0–3] correlates with a lower frequency of thrombosis, a higher frequency of palpable splenomegaly and more frequent progression to post polycythaemia myelofibrosis.[52]

Essential thrombocythemia

Essential thrombocythemia[ET] is a disease resulting from the clonal proliferation of a multipotent myeloid stem cell but with the predominant disease features resulting from increased platelet production. ET is characterized by a marked thrombocytosis [usually 1000–4000 × 10^9/L] often resulting in hemorrhagic or thrombotic episodes or both. Essential thrombocythemia is a myeloproliferative neoplasm that affects relatively young individuals and is characterized by markedly elevated platelet count but relatively low thrombotic risk.[52]

At least half of WHO-defined cases are asymptomatic and are diagnosed from an incidental blood count. About two thirds of symptomatic patients suffer venous or arterial thrombosis or symptoms attributable to small vessel obstruction, such as headache, dizziness, visual disturbance, paresthesia and peripheral vascular insufficiency, bleeding abnormalities. [chapter 5, bone marrow pathology; Barbara bain] [54]

Major thrombotic complications splenic or hepatic vein thrombosis as seen in Budd-Chiari Syndrome are less common in patients with a *CALR*

mutation than in patients with JAK2V617F. About a third of symptomatic patients have abnormal bleeding, for example into the gastro-intestinal tract or subcutaneous tissues.[35]

WHO-defined ET cases harbor a phenotypic driver mutation in JAK2[present in 50-60% of cases], CALR[in 30%], or MPL[in 3%], about 12% of cases are triple-negative for these mutations. None of these mutations is specific for ET, but their presence does exclude reactive thrombocytosis. [chapter 2, WHO classification of hematopoietic and lymphoid tissue.[35]

On peripheral blood film examination-there is increased number of platelets with giant platelets, agranular and hypogranular platelets. Mild leukocytosis with neutrophilia and occasional immature granulocytes may be present but the total leucocyte count does not usually exceed 20 × 10^9/L. The 2008 WHO classification and its 2016 revisionrequire a sustained platelet count of at least 450× 10^9/L to establish the diagnosis.Previously, a count of at least 600 × 10^9/L was required but a lower cut-off point is important forrecognition of a larger group of patients who mayhave thrombotic complications, splenomegaly andtypical histological features before reaching theprevious threshold.[55]

On bone marrow aspirate-normocellular marrow is seen in most cases. There is an increase inmegakaryocytes, which are large and hyperlobated. Leucopoiesis and erythropoiesis if increased is usually mild. On bone marrow histology marrow fragments are normocellular or occasionally mildly hypercellular. Megakaryocytes are increased in number in all cases. The size of megakaryocytes is increased, with hyperlobated forms referredto as staghorn-like and they are believed to represent hypermature megakaryocytes.

Cytogenetic- No molecular genetic abnormality is specific for ET. Approximately 50-60% of WHO-defined ET cases carry JAK2V617F, about 30% of cases have a mutation in CALR and 3% MPL. [chapter 2, WHO classification of hematopoietic and lymphoid tissue.[35] These

mutations are not specific for ET, they are also found in polycythemia vera and primary myelofibrosis. Cases with a CALR mutation are distinct from JAK2-mutated cases with a lower thrombotic risk, no risk of transformation to PV and a lower risk of transformation to acute leukemia.

In comparison with JAK2 mutation, CALR mutation is associated with younger age, male gender, higher platelet count, lower Hb and lower WBC. Survival does not differ between JAK2V617F and CALR-mutated ET.[53]

Diagnostic criteria of the 2016 revision requires that either all major criteria or the first 3 major criteria plus the minor criterion

Major criteria

1. Platelet count more than or equal to 450 x 10^9 /L
2. Bone marrow biopsy showing proliferation mainly of the megakaryocytic lineage, with increased numbers of enlarged, mature megakaryocytes with hyperlobulated nuclei, no significant increase or left shift in neutrophil granulopoiesis or erythropoiesis, very rarely a minor [grade 1] increase in reticulin fibres
3. WHO criteria for BCR-ABL1- positive chronic myeloid leukemia, polycythemia vera, primary myelofibrosis, or other myeloid neoplasms are not met
4. JAK2, CALR, or MPL mutation

Minor criterion

1. Presence of a clonal marker
2. Absence of evidence of reactive thrombocytosis

Diagnostic criteria for post-essential thrombocythemia [ET] myelofibrosis

Required criteria

1. Documentation of a previous diagnosis of WHO-defined ET

2. Bone marrow fibrosis of grade 2 to 3 on a 0-3 scale or grade 3-4 on a 0-4 scale

   Additional criteria [2 are required]

1. Anemia [i.e. below the reference range given age, sex, and altitude considerations] and a >2 g/dL decrease from baseline hemoglobin concentration
2. Leukoerythroblastosis
3. Increasing splenomegaly, defined as either an increase in palpable splenomegaly of5 cm from baseline [distance from the left costal margin] or the developmentof a newly palpable splenomegaly
4. Elevated lactate dehydrogenase level [above the reference range]
5. Development of any 2 [or all 3] of the following constitutional symptoms: 10% weight loss in 6 months, night sweats, unexplained fever[> 37.5 °C

Distinguishing ET from reactive thrombocytosis- Peripheral blood film is useful having giant platelets, some agranular platelets and occasionally circulating megakaryocyte nuclei. In reactive thrombocytosis the platelets are small and normally granulated and circulating megakaryocyte nuclei are not seen. The bone marrow aspirate and trephine biopsy sections often show an increase in megakaryocyte number and size but megakaryocytes are cytologically normal; they do not occur in large clusters orclose to the endosteum .[54]

Distinguishing ET from other MPNs causing thrombocytosis:

Polycythemia vera- shows trilineage hyperplasia

- Megakaryocytes increased in number in loose clusters with pleomorphic megakaryocytes with variable size, hyper and hypolobated forms.

- Reticulin fibres normal or slight increase with no increase in collagen fibres and no osteosclerosis

Primary myelofibrosis, prefibrotic-

- Increased cellularity
- Dense megakaryocytic clusters with atypical, cloud like, balloon like and bare megakaryocytes
- Reticulin fibres normal or slight increase with no increase in collagen fibres and no osteosclerosis

Primary myelofibrosis, fibrotic-

- Normal to hypocellular marrow
- Large clusters or sheets of megakaryocytes with atypical megakaryocytes
- Markedly increased reticulin fibres with presence of collagen and osteosclerosis.

Patients with platelet counts below the limit of 600 × 109 /l often presented with typical ET-related complications leading to diagnostic evaluation but no prognostic relevance of the initial platelet level on survival and symptom-free survival was seen and severity of complications did not depend on a definite platelet count.

In asymptomatic disease the rate of increase of symptomatic cases during follow-up was similar and independent of an initial platelet count above or below 600 × 109 /l [55]

The risk of thrombosis was similar in JAK2-mutant essential thrombocythemia and in polycythemia vera, while it was much lower in CALR-mutated essential thrombocythemia. These observations are consistent with the notion that JAK2 [V617F]-mutant essential thrombocythemia and polycythemia vera are different phenotypes in the evolution of a single neoplasm, while CALR-mutant essential thrombocythemia is a distinct entity.[52]

Primary myelofibrosis

Primary myelofibrosis [PMF] is a clonal myeloproliferative neoplasm [MPN] characterized by a proliferation of predominantly abnormal megakaryocytes and granulocytes in the bone marrow, which is associated with reactive deposition of fibrous connective tissue and with extramedullary hematopoiesis.

There is a stepwise evolution from an in initial prefibrotic/early stage[31] characterized by hypercellular bone marrow with absent or minimal reticulin fibrosis, to an overt fibrotic stage with marked reticulin or collagen fibrosis and often with osteosclerosis. Myelofibrosis is indistinguishable from PMF. Myelofibrosis may develop following PV and ET. CML can also evolve into myelofibrosis. Cases with preceding PV or with an increased red cell mass are classified as post-polycythaemic myelofibrosis. Cases with previous ET are classified as post-ET myelofibrosis. Cases with Philadelphia or BCR-ABL1 positivity represent evolution of CML, usually accelerated phase or blast transformation. [35]

Overt primary myelofibrosis [PMF] is an MPN characterized by splenomegaly, a leucoerythroblastic anemia and marrow fibrosis. It is a chronic disorder in which patients may remain relatively asymptomatic until the later stages and are discovered by presence of splenomegaly during a routine physical examination or when a routine blood count shows anemia, leukocytosis, or thrombocytosis. Less commonly, the diagnosis results from discovery of unexplained leukoerythroblastosis or an increased lactate dehydrogenase level. Mild or moderate hepatomegaly is also common.[54]

Clinical presentation- Patients experience constitutional symptoms such as fatigue, dyspnoea, weight loss, night sweats, low-grade fever, and cachexia.

On peripheral blood film- PMF shows pancytopenia, a leucoerythroblastic blood film and poikilocytosis including teardrop poikilocytes. Granulocytes and platelets may show dysplastic features

such as hypolobation of neutrophil nuclei, reduced granulation of eosinophils or large or hypogranular platelets

Bone marrow aspiration- due to fibrosis aspiration is often difficult. In the early stage of the disease, aspirate is possible to obtain and shows very hypercellular fragments with an increase in cells of all lineages with normal maturation.[56,57] In the later stages of the disease, there is often failure to obtain an aspirate [a 'dry tap'] or aspiration yields blood [a 'blood tap']. Diagnosis then is made on the peripheral bloodand trephine biopsy histology findings.[54]

Prefibrotic/early primary myelofibrosis

Thebone marrow biopsy shows hypercellularity with an increase in the number of neutrophils and atypical megakaryocytes. Erythropoiesis is reduced. The megakaryocytes are markedly abnormal and atypical. Topographical distribution of megakaryocytesis critical for the recognition of pre-PMF.[58]

There are dense clusters of megakaryocytes and are adjacent to the bone marrow vascular sinuses and bone trabeculae. Most cases of pre-PMF eventually transform into overt fibrotic myelofibrosis associated with extramedullary hematopoiesis.

Overt primary myelofibrosis

It is associated with dense and diffuse collagen fibrosis and osteosclerosis with small islands of hematopoietic elements situated mostly with in the vascular sinuses. There is significant proliferation of vessels having marked tortuosity and luminal distension.

Atypical megakaryocytesare conspicuous finding and occur in large clustersor sheets often within dilated vascular sinuses.[59] As the degree of fibrosis increases, granulocytic and erythroid precursors in the marrow decrease.

The morphological abnormalities in the megakaryocytic series become more prominent. In severely fibrotic marrows there is fibroblast proliferation and collagen deposition. Sinusoids may be obliterated by progressivefibrosisbut capillaries are very numerous.[60]

Cytogenetics- No genetic defect specific for PMF has been identified. Approximately 50-60% of WHO-defined PMF cases carry JAK2V617F or a functionally similar mutation, about 30% of cases have a mutation in CALRand 8% in MPL [chapter 2, WHO classification of hematopoietic and lymphoid tissue][35]

Although the JAK2mutation confirms the clonality of the proliferation, the mutation is also found in polycythemia vera and essential thrombocythemia, and thus does not distinguish PMF from these MPNs. A study using multivariate analysis found an Hb less than 100 g/l, a WBC of 20 × 10^9/L or more and a platelet count of 300 × 10^9/L or less to be associated with a worse prognosis.[61]

CALRmutation has been associated with a lower risk of anemia and thrombocytopenia, marked leucocytosis and thrombosis.[53]

In comparison with JAK2V617F cases, those with a CALR mutation tend to be younger and have a higherplatelet count and a lower WBC.[62]

Chronic neutrophilic leukemia

It is a rare myeloproliferative neoplasm characterized by sustained peripheral blood neutrophilia, bone marrow hypercellularity due to neutrophilic granulocyte proliferation and hepatosplenomegaly. There is no Philadelphia [Ph] chromosome or BCR-ABL1 fusion gene.

Diagnostic criteria for chronic neutrophilic leukemia

1. Peripheral total leucocyte count >25 x 10^9/L

- Segmented neutrophils plus banded neutrophils > 80% of the white blood cells

- Neutrophil precursors [promyelocytes, myelocytes and metamyelocytes] constitute< 10% of the white blood cells
- Myeloblasts rarely observed
- Monocyte count< 1 x 10^9/L
- No dysgranulopoiesis

2. Hypercellular bone marrow
- Neutrophil granulocytes increased in percentage and number
- Neutrophil maturation appears normal
- Myeloblasts constitute < 5% of the nucleated cells

3. Not meeting WHO criteria for BCR-ABL1-positive chronic myeloid leukemia, polycythemia vera, essential thrombocythemia or primary myelofibrosis

4. No rearrangement of PDGFRA, PDGFRB, or FGFR and no PCM1-JAK2 fusion

5. CSF3R T6181 or another activating CSF3R mutation

Or persistent neutrophilia present for more than 3 months with splenomegaly and no identifiable cause of reactive neutrophilia including absence of a plasma cell neoplasm. If present demonstration of clonality of myeloid cells by cytogenetic or molecular studies.

On peripheral blood film examination- It shows neutrophilia with leukocytosis, TLC >25 x 10^9/L and increase in mature neutrophils and band forms without any eosinophilia or basophilia and with very few neutrophil precursors. The peripheral blood shows an increase in mature neutrophils and band forms without any eosinophilia or basophilia and with very few neutrophil precursors. There is no increase in reticulinfibres.[63] Abnormal localization of immature neutrophil precursors is sometimes seen and megakaryocytes may be atypical with some clustering.[64]

Bone marrow aspiration- shows hypercellular marrow with an increase in myeloid series with no increase in blasts.

Bone marrow biopsy- Bone marrow biopsy shows hypercellularity, with neutrophilic proliferation. The M:E may reach >20:1.

Cytogenetic- normal in nearly 90% of cases.The remaining cases, reported clonal karyotypic abnormalities include gains of chromosomes 8, 9, and 21; del[7q]; del[20q]. There is a strong association with *CSF3R* T618I or other activating mutation of CSF3R[63]

Chronic eosinophilic leukemia, not otherwise specified.

Itis a subtype of CML with differentiation towards eosinophils. There isincreased numbers of eosinophils in the peripheral blood, bone marrow, and peripheral tissues.

Clinical presentation- can includes hepatosplenomegaly and anemia along with other constitutional symptoms. There may be tissue damage due to release of contents of eosinophil granule.

Diagnostic criteria for chronic eosinophilic leukemia, NOS

1. Eosinophilia [eosinophil count >1.5 x 10^9/L]
2. WHO criteria for BCR-ABL1-positive chronic myeloid leukemia, polycythemia vera, essential thrombocythemia, primary myelofibrosis, chronic neutrophilic leukemia, chronic myelomonocytic leukemia and BCR-ABL1-negative atypical chronic myeloid leukemia are not met
3. No rearrangement of PDGFRA, PDGFRB or FGFR1, and no PCM1-JAK2, ETV6-JAK2, or BCR-JAK2 fusion
4. Blast cells constitute <20% of the cells in the peripheral blood and bone marrow and inv[16][p13.1q22], t[16;16][p13.1;q22], t[8;21][q22;q22.1], and other diagnostic features of acute myeloid leukaemia are absent
5. There is a clonal cytogenetic or molecular genetic abnormality or

Blast cells account for >2% of cells in the peripheral blood or >5% in the bone marrow

Peripheral blood film -shows eosinophilia with small numbers of eosinophilic myelocytesand promyelocytes. The eosinophils may be cytologically normal or may show abnormalities such as degranulation, cytoplasmic vacuolation, hypolobation or hyperlobation.

The bone marrow aspiration and biopsy- shows hypercellularity with prominence of eosinophils and eosinophilic precursors with orderly maturation. Charcot-Leyden crystals are often seen as well. Differential of chronic eosinophilic leukemiasis idiopathic hypereosinophilic syndrome, The CEL, NOS is a diagnosis of exclusion, when there is no potential cause of reactive eosinophilia. The patientsshould be investigated for underlying causes of eosinophilia and should have cytogenetic and molecular genetic analysis. It is essential to investigate for a FIP1L1-PDGFRA fusion gene.[35]

Cytogenetics- No single or specific cytogenetic or molecular genetic abnormality has been identified in CEL, NOS. The t[9;22];BCR-ABL1, rearrangement of PDGFRA, PDGFRB or FGFR1,PCM1-JAK2 fusion and inv[16];CBFB-MYH11 are specifically excluded.[22]

The JAK2 V617F mutation is found in occasional patients with otherwise unexplained hypereosinophilia, permitting a diagnosis of CEL.[23] Occasional patients have a CBL mutation.[24] Occasional patients with CEL, NOS have cytologically normal eosinophils, but the absence of eosinophil dysplasia generally favors reactive eosinophilia.[65]

Myeloproliferative neoplasm, unclassified

Diagnostic criteria for myeloproliferative neoplasm [MPN], unclassifiable.

All three criterias needs to be present for diagnosis:

1. Features of an MPN are present
2. WHO criteria for any other MPN, myelodysplastic syndrome", myelodysplastic/ myeloproliferative" neoplasm, or BCR-ABL1-positive chronic myeloid leukemia are not met

3. JAK2, CALR, or MPLmutation characteristically associated with MPN or presence of another clonal marker or absence of a cause of reactive fibrosis

The MPN-Uisdiagnosed only when there is definite clinical, laboratory, molecular, and morphological features of a myeloproliferative neoplasm [MPN] but the diagnostic criteria for any of the specific MPN entities is not met or if the presentation overlap between two or more of the MPN categories.

Most cases of MPN-U fall into one of three groups: [chapter 5, WHO classification of hematopoietic and lymphoid tissue, 2016][35]

1. Subset of cases with masked pre-polycythemic presentation of polycythemia vera, prefibrotic/early primary myelofibrosis, or early-phase essential thrombocythemia when the characteristic features are yet fully developed
2. Advanced-stage MPN, when pronounced myelofibrosis, osteosclerosis, or transformation to a more aggressive stage with increased blast counts /myelodysplastic changes obscures the underlying disorder
3. Cases with evidence of an MPN but with coexisting neoplastic or inflammatory disorder obscuring some of the usual diagnostic clinical and morphological features.

Clinical presentation- is similar to other entities of MPN. Patients present with anemia, fatigue, weight loss, unexplained fever, malaise, loss of appetite, organomegaly. Peripheral blood smears - shows thrombocytosis and variable neutrophilia.[66] The hemoglobin varies from normal to increased to decreased.

Bone marrow biopsy findings- show hypercellularity with prominent megakaryocytic proliferation. In late-stage there is dense fibrosis or osteomyelosclerosis, indicating a terminal burnt-out stage.[35]

Cytogenetics- there is no specific cytogenetic for MPN-US. The presence of BCR-ABL 1 fusion; rearrangement of PDGFRA, POGFRB, or FGFR1; or PCM1-JAK2 fusion excludes the diagnosis of MPN-U.[67]
This diagnosis is also excluded if clinical data required for proper classification are not available, bone marrow specimen is inadequate for evaluation, patient who recently received cytotoxicor growth factor therapy. Cases with prominent cytopenia or significant accompanying myelodysplastic changes are categorized as myelodysplastic/myeloproliferative neoplasm [MDS/MPN][68,69,70].

F.A. Bhatti, et.al 2012 analyzed the data of patients suffering from 425 patients of MPN. Out of 425 patients suffering from myeloproliferative neoplasm, 335 [79%] patients were diagnosed as chronic myelogenous leukemia. Majority of patients with CML were in the chronic phase of the disease [n = 240, 71.6%], with 49 [14.6%] in the accelerated phase, 31 [9.3%] in the myeloid blast crisis. Age of the patients ranged from 2–80 years [median: 42 years], with a male to female ratio of 2:1 [225:110]. Peak incidence of the disease was seen between 21–50 years. 14 patients [5.8%] in chronic phase of CML were asymptomatic and were discovered incidentally. The duration of symptomatic disease ranged from 1 week to 3 years, with maximum number of patients [n = 225; 67%] reporting within 3 months of the onset of symptoms. The most common clinical presentations was anemia [63.6%] including symptoms of fatigue, breathlessness, dizziness, lethargy, and body aches and only 27[8%] had Hb>12g/dl, and splenomegaly in 158 [47.2%] presenting with abdominal distension, pain in left hypochondrium, and dragging sensation in abdomen. Thrombocytosis was seenin 88 [26%] patients in all phases of CML. [71]

Vytrva, Nataliya. et.al 2014 compared 112 BM biopsies obtained from patients with Ph negative MPNs. JAK2 V617F mutation was detected in

24 of 52 ET cases [46.2%], 36 of 38 PV cases [97.5%], and 14 of 22 PMF cases [63.6%].Morphology of megakaryocytes in bone marrow biopsies was assessed and correlated with clinical and laboratory data. In multivariate analysis, higher hemoglobin concentration, higher white blood cell counts, and lower platelet counts were significantly associated with JAK2 V617F. Striking morphologic similarities were found between polycythemia vera JAK2 V617F and primary myelofibrosis JAK2 V617F indicating that primary myelofibrosis JAK2 V617F and polycythemia vera JAK2 V617F sharesame pathogenetic pathways, resulting in morphologically similar megakaryocytes.[72]

Lesley A. Anderson, 2015. Studied the symptom burden on patients with MPN.106 patients were analysedand fatigue was the most common symptom [92.4%]. Female MPN patients suffered worse symptom burden than male patients. The cough was significantly higher in PMF patients than PV [P = 0.039] or ET [P = 0.024] patients. Fever was also more frequent in PMF than PV patients. Patients with PMF reported the worst symptom burden [88.3%].[73]

S. Varma, MD, et.al 2015 conducted a hospital based study with a total of 298 patients. Out of which 231 were diagnosed as MPN. Of the 231 MPN patients, 207 [89.6%] were BCR-ABL positive CML. The remaining 24 [10.4%] patients were BCR-ABL negative MPN; most common was PV diagnosed in 11 [4.7%], followed by PMF in 7 [3%] and ET in 6 [2.6%] cases. Molecular analysis of BCR-ABL negative MPN revealed JAK2V617F positivity in 9/11 [81.8%] cases of PV, 7/7 [100%] cases of PMF and 2/6 [33.3%] cases of ET.[74]

Belinda B. Guo, et.al 2017 in a study examined the megakaryocyte genome in 12 patients with MPNs to determine whether there are somatic variants and association with marrow fibrosis. 6 of the 12 patients had increased reticulin content [grade 2]. 9 patients were JAK2V617F positive

and 3 were triple-negative [JAK2V617F, CALR, and MPL negative]. Targeted next-generation sequencing was done for 120 genes associated with myeloid neoplasms, on megakaryocytes. 10 of the 12 patients had genomic defects in megakaryocytes that were not present in nonmegakaryocytic hemopoietic marrow cells from the same patient. The greatest allelic burden was in patients with increased reticulin deposition. The megakaryocyte-unique mutations were predominantly in genes that regulate chromatin remodeling, chromosome alignment, and stability. These findings show that genomic abnormalities are present in megakaryocytes in MPNs and that these appear to be associated with progression to bone marrow fibrosis.[75]

Tahlan A, et al. 2017 retrospectively assessed the hematological and parameters and bone marrow histomorphology in 120 cases of patients with MPNs. 97 patients diagnosed with chronic myeloid leukemia [CML]; 6 patients with polycythemia vera[PV]; 5 patients with essential thrombocythemia[ET]; 11 with Primary Myelofibrosis [PMF]; and 1 with chronic neutrophilic leukemia [CNL]. Maximum number of cases of MPNs occurred in the 51-60 age group with fever presenting as the most common clinical symptom, followed by abdominal problems. Mean age of MPN presentation was 40.7 years[76]

Suchi Gha et. al. [2018] in a study of 44 cases diagnosed as MPN on complete blood count, peripheral blood examination, BMA and BMB with cytogenetic mutation study out of which 24 cases [54.4%] of chronic myeloid leukemia, 7 cases [46.6%] of PV, 3 cases [6.8%] of ET, 5 cases [11.4 %] were of MF and 5 cases [11.4%] of myeloproliferative neoplasm-unclassified. Commonest age group affected was between 40 to60 years in all disease categories. Males were affected predominantly in all the five disease entities with M: F ratio of 1.1:1 in CML, 6:1 in PV, 2:1 in ET, 3:2 in MF and 4:1 in MPN-U. Weakness was the most common

symptom in CML [95.8%]. Significant peripheral smear findings included red cell crowding in all cases [100%] of PV cases. Basophils were present in all the cases of MPN in peripheral smears studied.In BMA and BMB increased cellularity and panmyelosis was seen in all cases [100%] of PV, ET, MPN-U. Myelofibrosis revealed normal to increased cellularity in 3 cases [60 %] and decreased cellularity in 2 cases [40 %]. Dry tap was noted in 2 cases [40 %].[77]

In 2017 a review study by the Mumbai Cancer Registry specifically examined CML from period of 1990 to 2016 reviewed 37 studies containing > 200 patients and reported median age of 35 years, M:F ratio of 1.6:1, <5% patients were asymptomatic, constitutional symptoms in 60% of patients, splenomegaly in 95% patients. The incidence varied across age groups, with an increased incidence in older individuals. Interestingly, there was a decreasing trend in the incidence of CML over the years[78]

Motohiko Okano, et. al, 2017. In a review study of 11 CML cases with marked thrombocytosis and without significant leukocytosis, which may be misdiagnosed as ET. Most of them were young females without immature myeloid cells in peripheral smear. 2 out of 11 patientsin their thirties had remarkable thrombocytosis, over $3{,}000\times10^3/\mu l$, and without significant leukocytosis. Although this finding resembled that of essential thrombocythemia [ET], but the patients were diagnosed with CML because of the presence of Philadelphia chromosome. JAK2, CALR, and MPL were unmutated on fluorescence in situ hybridization analysis. We reviewed. These findings suggested that there is a subgroup of CML patients.[79]

Pereira A, et.al 1989 studied the clinical and hematological profile of 53 patients in whom primary myelofibrosis [PMF]. Median age was 64 years

[range 17-86]. 35 patients were males and 18 females. The most frequent symptoms were associated with the hypermetabolic state, anemia and splenomegaly. The splenomegaly was found in 96% of patients, while 83% had hepatomegaly and 9% had lymphadenopathy. 33 patients had anemia at the time of diagnosis. The leukocyte and platelet counts were normal or moderately high in most cases. Myelemia was found in 83% of patients, with circulating erythroblasts in 72%. The most common biochemical abnormalities were the increased serum LDH [84%] and hypocholesterolemia [62%]. Bone marrow aspirate was not analyzable in most cases. Bone marrow biopsy showed myelofibrosis in hypercellularphase in 22 patients, myelofibrosis without osteosclerosis in 17, and myelofibrosis with osteosclerosis in 14. The median survival was 3.8 years; 34 patients had died at the time of the analysis. The major causes of death were infection, cardiovascular complications and hemorrhage. In 4 patients the evolution from PMF to acute leukemia was observed.[80]

Huang J, et.al 2008 In a study of 311 patients with PMF studied independent risk factors for leukemic transformation which included circulating blast > 3% and platelet count < 100x10^9 /L. The rate of leukemic transformation was only 6% in the absence of both risk factors and 18% in the presence of one or both risk factors.[81]

Tefferi et al. 2014 studied 254 patients with PMF and reported mutational frequencies of 58% for JAK2, 25% CALR, 8% MPL, and 9% wild-type for all three mutations [i.e., triple-negative]. CALR mutational frequency in JAK2/MPL-unmutated cases was 74%. CALR mutations were associated with younger age, higher platelet count. CALR-mutated patients were also less likely to be anemic, require transfusions, or display leukocytosis.[53]

Sadia Sultan., et.al 2015 in a retrospective cross sectional study of 20 patients with idiopathic myelofibrosis studies the clinical and symptomatic presentation in male sand females. The mean age was 57.9±16.5 years with 70% of patients aged above 50. The M:F was 3:1.The majority of PMF patients were male and presented with constitutional symptoms.Only 10% of patients were asymptomatic and the rest presented with constitutional symptoms. In symptomatic patients, major complaints were weakness [80%], weight loss [75%], abdominal discomfort [60%], night sweats [13%], pruritus [5%] and cardiovascular accidents [5%]. Splenomegaly as a predominant finding detected in 17 patients [85%]. The mean age was 57.9±16.5 years with 70% of patients aged above 50. The male to female ratio was 3:1. Overall only 10% of patients were asymptomatic and the remainder presented with constitutional symptoms. The mean hemoglobin was 9.16±2.52 g/dl. The total leukocyte count of 17.63±19.17 x10^9/L and platelets count were 346.50±321.93x10^9 /L. 13 [65%] patients were anemic and 4 [20%] patients had thrombocytopenia. Leucopenia and leukocytosis were seen in 5 [25%] and 4 [20%] cases respectively.[82]

Adalet Meral Gunes, et.al 2018 studiedpediatric CML patients from age 0-18 years registered from 2010 until 201. 22/479 patients [4.6%, ratio male/female: 14/8] were studied with a median age of 22 month. Median spleen size was 12 cm [2-20] and median liver size was 3 cm [2-6] below the costal margins. Major presenting complains and symptoms were asthenia [30%], fever [30%], abdominal pain [20%], extramedullary signs [14%], hemorrhage [5%], and weight loss [5%]. Median WBCwas 154 600/µl [23 000-892 000], median Hb was8.7 g/dl [5.6-11.5g/dl] and platelet counts 311 000/µl [51 000-1 820 000]. Philadelphia chromosome and BCR-ABL1 gene rearrangement were detected by cytogenetic and/or molecular techniques [FISH and RQ-PCR][83]

D Barraco, et.al, 2016 in a study of 722 PMF patients [median age was 64, and 64% were males] stratified the patients according to the grade of anemia as mild [hemoglobin level >10 g/dl but < sex-adjusted normal value], moderate [hemoglobin level between 8 g/dl and <10 g/dl] and severe [hemoglobin <8 g/dl or transfusion-dependent]. 628 [87%] patients had anemia, of which 295 [47%] were mild, 98 [16%] moderate and 235 [37%] severe. In univariate analysis, anemia was significantly associated with advanced age, lower leukocyte count, lower platelet count, presence of circulating blasts, presence of constitutional symptoms, driver mutational status.[84]

In a study by Lengfelder E, et al. 1998; clinical data and prognostic factors of 143 patients with ET were retrospectively analyzed. In 42 patients the early phase of the disease with initial platelet counts between 250 to 600×10^9/L was assessed. In most early cases, ET was suggested by clinical symptoms [79%] and increased megakaryopoiesis [95%] with abnormal megakaryocytes on bone marrow examination. During follow-up of the 38 early cases not treated cytoreductively at diagnosis, the platelet counts increased to >600x10^9/L in 28 patients [74%] and remained between 450 to 600x10^9/L in 10 patients [26%]. No influence of the initial platelet count on survival was seen in multivariate analysis of prognostic factors which included all 143 cases. Survival was mainly influenced by the rate of ET-related complications during follow-up.[55]

Barbui T, et.al. 2011 studied clinical course of ET in the series of 1,104 patients and compared with prePMF. Amongst 1104 cases 891 patients [81%] were of ET and 180 [16%] were prePMF. A comparative analysis of clinical and laboratory data for patients with ET and prePMF showed that age and gender distribution and JAK2V617F mutational frequencies were similar between these two groups. Significant differences were seen for leukocyte count, hemoglobin level, platelet count, serum lactate

dehydrogenase [LDH] level, incidence of palpable splenomegaly and circulating CD34 cell count, all greater in prePMF than ET. The results of study indicate that the distinction between [true] ET and prePMF is of significant importance concerning the survival rates, leukemic transformation rates, and rates of progression to overt myelofibrosis that were significantly worse in prePMF than in ET [74]

AyalewTefferi, et.al, 2016 in a study of patients with ET [n= 576]. Patients with CALR-mutated patients displayed younger age, male sex, higher platelet count, lower hemoglobin level, lower leukocyte count, and lower incidence of thrombosis. Triple-negative ET patients also displayed younger age, lower hemoglobin level, lower leukocyte count, and lower incidence of thrombosis, compared with JAK2-mutated cases. Comparison of triple-negative, CALR mutated and ET patients showed the latter to be associated with male sex and higher platelet count. MPL-mutated cases were similar to JAK2- mutated cases in terms of age and gender distribution and thrombosis risk but displayed lower hemoglobin level.[53]

In study by Tereffi A. et.al, 2014, compared clinical and laboratory features of 826 patients. Cases with a CALR mutation are distinct from JAK2-mutated cases with a lower thrombotic risk, no risk of transformation to PV and a lower risk of transformation to acute leukemia. In comparison with *JAK2* mutation, CALR mutation is associated with younger age, male gender, higher platelet count, lower Hb and lower WBC. The expected differences in hemoglobin level and platelet count, the other significant differences between ET, PV, and PMF included younger age in ET, predominance of male sex in PMF, and higher leukocyte count in PV.[53]

In a study performed by Barbui T., et al, 2014.amongst 397 JAK2V617F and/or Exon 12-mutated patients with strictly WHO-defined PV

morphology of which 257 [65%] met the full WHO-2008 criteria for diagnosis of PV. The remaining 140 patients [35%] were classified as so called masked PV based on levels of hemoglobin [Hb] ranging from 16.0 to 18.4 g/dL in men and from 15.0 to 16.4/dL in women and frequent presence of subnormal erythropoietin levels. These patients were more males, had more previous arterial thrombosis and presented more frequently with platelet counts exceeding the required level of WHO-defined ET [450x10^9/L]. The recognition of these prodromal PV patients has an important clinical impact since, in spite of not meeting the full WHO criteria, they present with same vascular risk as overt PV. Thus, the same therapeutic recommendations given for overt PV should be reasonably applied.[85]

MATERIAL AND METHODS

Source of data

This study was conducted for a period of 5years both prospectively and retrospectively in the Department of Pathology, Dayanand Medical College and Hospital, Ludhiana. All cases diagnosed with myeloproliferative neoplasms during the study period, as per criteria for diagnosis in WHO Hematopoietic and lymphoid tissues, 2017 revised edition were analyzed in regard to clinical features, hemograms, bone marrow aspiration and biopsyfindings and cytogenetics.

Inclusion criteria

All the diagnosed cases of myeloproliferative neoplasms whose bone marrow aspirations and biopsy have been done in department of pathology.

Duration of Study

This study was conducted over a period of five year [3.5 years retrospective; 1st April 2016 – 30th September 2019] and 1.5 years prospective; 1st October 2019 – 31st March 2021]

Study Design

This was a Prospective and Retrospective study

Methodology

Clinical presentations with hemogram, bone marrow aspiration and biopsy findings and molecular cytogenetics were analyzed and recorded.

The following investigations were performed and recorded in all cases:

1. Complete Hemogram

Hemoglobin, total leucocyte count, differential leucocyte count , platelet count, hematocrit and Red blood cell count[by automated cell counters and cross checked manually].

2. Peripheral smear examination was done after Giemsa staining.

3. Bone Marrow Aspiration

Needle: Salah marrow puncture needle

Site: Posterior superior iliac spine

Procedure:

The site was prepared with betadene and spirit. The skin, subcutaneous tissue and periosteum overlying the skin of aspiration were infiltrated with a local anaesthestic[lignocaine 2%]. The guard was adjusted according to the depth of the outer table of the bone from the surface. The skin and subcutaneous tissue were punctured with the needle and then with a boring motion, the needle was pushed into the marrow cavity.The stillette was removed and few drops of marrow sucked with a 20 cc syringe.The marrow was then expelled out onto the slides, excess blood drained by tilting the slides and smears prepared.

Staining: Giemsa

Both peripheral blood film and marrow aspirates were stained with giemsa stain.

Procedure for Giemsa staining– Air dried smears were fixed in methanol for 5-15 minutes, flooded with freshly prepared buffered Giemsa stain [in 10 dilution] and kept for 15 minutes. Then they were washed with buffered water and dried.

4**. Bone marrow biopsy**

Needle used - Jamshidi needle

Site- Posterior superior iliac spine

Procedure: Under all aseptic procedures and using anesthesia [2% lignocaine], biopsy was performed from posterior iliac crest. It was done along with aspiration in the same setting. Following aspiration, the stilette was withdrawn from the needle and then the cap was closed. Now it was further pushed into the cavity by rotating movements for about 0.5-1 cm. The process captures the marrow core sample within the needle. The needle was then withdrawn in the reverse rotating direction. A wire probe was inserted at the hub of needle on to a sterile gauge. Contact smears were made by rolling the cylindrical marrow biopsy strip on the slide.

Processing: The biopsy was placed in formalin for 14-24 hours and washed for at least six hours. It was then processed through graded

alcohols and acetone on the automatic tissue processor. After embedding in paraffin wax sections of 5-6µm thickness were cut.

Mercuric precipitates were removed using alcoholic iodine and sodium thiosulphate.

Staining- Hematoxylin & Eosin [H&E]

Procedure of Hematoxylin and Eosin Staining

- Slides were kept on hot plate in order to dewax the sections at temperature up to 60°C.
- Then slides were deparaffinised in xylene.
- Then hydration was done by passing through descending rates of alcohol to water.
- The slides were then stained in Harris Hematoxylin for 15 minutes. Sections were washed well in running tap water until sections turn blue. These sections were then differentiated in 1% acid alcohol [1% HCI in 70% alcohol] for 5-10 seconds, washed well in tap water till sections were again blue for 5 minutes or less.
- These were again stained in 1% Eosin for 10 min followed by washing in running tap water for 1-5 minutes.
- Sections were dehydrated once again through ascending grades of alcohol, cleaned in Xylene and mounted with DPX [distyrenedibutyl-phtalate xylene] avoiding air bubbles.
- Stained slides were then subjected to microscopic examination under both low [10x] and high power [40x].

5. **Molecular cytogenetics**

Fluorescence in situ hybridization:

- Steps- Denaturation: the probe and target DNA were denatured by incubation at high temperature.
- Hybridization: the probe was hybridized tothe chromosomal target.
- Washing: was done to remove theunbound probe.
- Analysis: was done under the fluorescence microscope. DNA was analyzed in cell on the chromosome and was not extracted or run in

a gel. In indirect FISH, biotin- ordigoxigenin-labeled nucleotides were visualized in by fluorescein isothiocyanate[FITC]-conjugated or CY3-conjugated avidin or rhodamine conjugated antidigoxigen in antibodies. Inthe direct method the probes were already labeled with fluorochromes.

- Procedure- For translocation detection, two probes were labeled with different fluorochromes. A normal cell displayed four signals [two of each color], while a cell with translocation showed two adjacent signals leading to a different colored fluorescence signal. The fusion of the signal was seen as a different color [e.g. yellow] which indicated the normal allele, whereas the two different signals [e.g. red andgreen] indicated the presence of translocation.
- The counter stain using propidium iodide [PI] orDAPI was used to identify chromosomes.

Reverse transcriptase- Polymerase chain reaction [RT-PCR]

- Sample-bone marrow aspirate [1–3 ml] samples in EDTA [purple top] tubes was processed within 8 hours of collection
- Sample preparation- RBC Lysis Solution was added bone marrow aspirate samples prior to RNA isolation by adding Qiagen RBC Lysis Solution. This was followed by Trizol reagent to extract total RNA, and after extraction the RNA samples were stored at −20°C and treated with DNase to remove possible DNA contamination of the sample
- RNA concentration was measured using a spectrophotometer and RNA concentration was adjusted in nuclease free H2O to a concentration of 0.2 mg/ml for subsequent cDNA synthesis
- Each reaction contained 6.2 ml of nuclease-free H2 O, 12.5 ml of 2× Taqman Universal PCR Master Mix, 0.15 ml of each forward and reverse primers [the final concentration of each primer be 300 nM], and 1 ml of TaqMan probe

- Centrifugation the reaction plate at low speed for 2 min was done, and then the plate was loaded onto an Applied Biosystems 7900HT real-time PCR instrument.

Next generation sequencing-

- Sample preparation- For DNA isolation, DNA polymerase was used to generate single stranded DNA template. A single primer complementary to template was added to initiate DNA synthesis.
- Sequencing –the DNA extracted were loaded on flow cell and placed on sequencer. All four nucleotides were added to sequencing chip and after nucleotide incorporation, the remaining DNA sequences were washed away. Each nucleotide contained a fluorescent tag and a reversible terminator that blocked incorporation of the next base. After reading the forward DNA strand, the reads were washed away, and the process was repeated for the reverse strand.
- Data analysis- analysis was read through the fluorescent emission as the DNA sequences generated during sequencing flow through the gel.

OBSERVATIONS AND RESULTS

This five year study [3.5 years retrospective;1st April 2016– 30th September 2019] and 1.5 years prospective ;1st October 2019 – 31st March 2021] was done in Department of Pathology, Dayanand Medical College and Hospital, Ludhianato analyze the hematological findings and genetic profile of myeloproliferative neoplasms. Myeloproliferative neoplasms were classified according to WHO classification of tumors of hematopoietic and lymphoid tissues, 2016.

Relevant data pertaining todemographic profile, clinical features, laboratory parameters and bone marrow aspiration and biopsy findings of all patients who were cytogenetically confirmed to have myeloproliferative neoplasm were recorded.

Of the 4438 bone marrow aspirations done, 138 [3.12%] were confirmed to have genetic mutational analysis of myeloproliferative neoplasm [CML, ET, PV, PMF] and were included foranalysis. The mutational analysis was confirmed by using qualitative/ quantitative Realtime polymerase chain reaction [RT-PCR], Fluorescence in situ hybridization [FISH], Next generation sequencing [NGS]. The cases were then categorized to BCR-ABL positive and BCR-ABL negative myeloproliferative neoplasms.

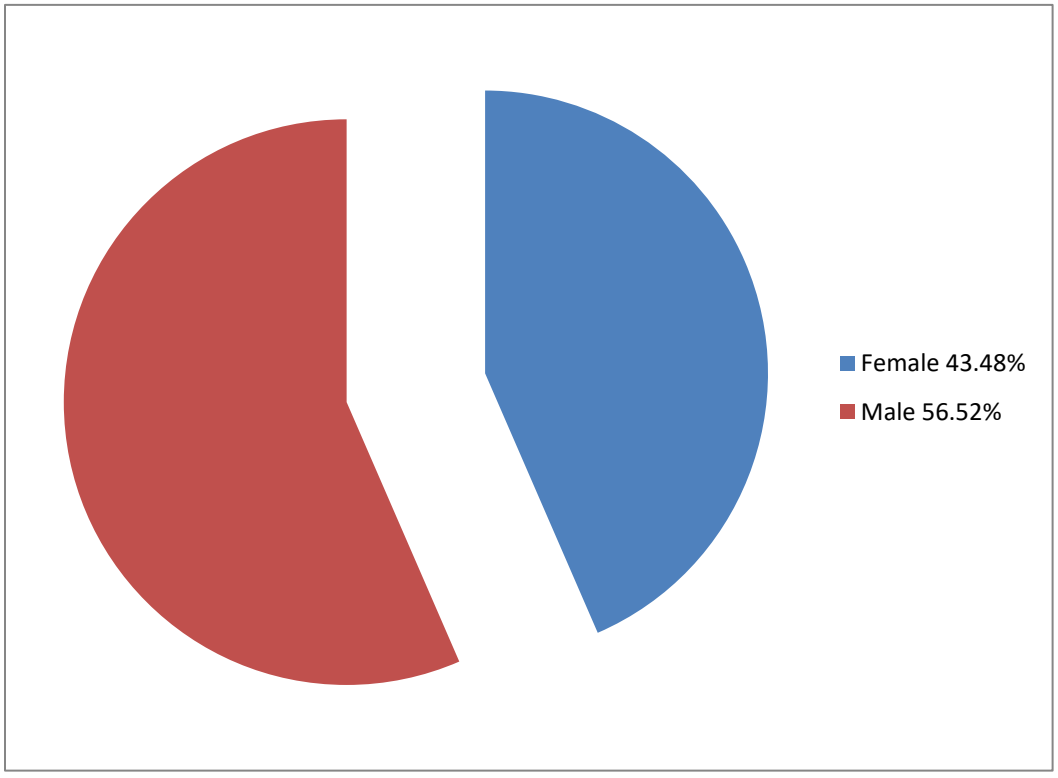
Age and gender wise distribution of all cases

Of all the 138 cases, majorityof the cases were seen in 41 to 50years of age group which included 35 cases [25.4%] followed by 61 to 70 years of age group [28 cases, 20.3%]. Mean age was 47 years. The youngest patient was 7 years old female and eldest was81 years male. Overall slight male predominance was noted with M:F was 1.3:1[60,43.48% females and 78, 56.52% males].[Table 1, Figure 1]

Table 1: Age wise distribution of patients

Age [years]	No. of cases	%
< 10	1	0.72
11-20.0	4	2.9
21-30	20	14.5
31-40	22	15.9
41-50	35	25.4
51-60	22	15.9
61-70	28	20.3
> 70	6	4.35

Figure 1: Gender wise distribution of patients



Molecular cytogenetics technique

Of the total 138 cases, 86 cases [62.30%] were confirmed by quantitative RT-PCR, 36 [26.20%] cases by RT-PCR qualitative, 14 [10.10%] cases by FISH and 2 [1.40%] cases were confirmed by NGS panel. [Table 2]

Table 2: Techniques used for confirmation of the type of myeloproliferative neoplasm

Type of technique	No. of cases	%
RT-PCR quantitative	86	62.30
RT-PCR qualitative	36	26.20
FISH	14	10.10
NGS	2	1.40
Total	138	100%

Distribution of cases according to frequency of mutation

Of the 138, 103 cases [74.64%] had BCR-ABL fusion gene,31 [22.46%] patients had negative BCR-ABL and positive JAK2v617f mutation and 4 [2.90%] patients had both BCR-ABL and JAK2 mutation. All 138 patients were negative for CALR and MPL mutations. [Table 3]

Table 3: Distribution of cases according to frequency of mutation

Type of mutation	No. of cases	%
BCR-ABL	103	74.64
JAK-2V617f	31	22.46
BCR-ABL, JAK2V617f	4	2.90
CALR	0	0
MPL	0	0

Distribution of cases based on type of myeloproliferative neoplasm

Most prevalent amongst 138 cases was Chronic myeloid leukemia [CML] constituting 105 cases [76.3%]. The cases with BCR-ABL negative and JAK2V617f was positive were analyzed, keeping in view thehemograms, bone marrow aspiration and biopsy findingsthese cases were categorized as ET,PV AND PMF. Following CML, second most common entity was Essential thrombocythemia [ET] 12 cases [8.63%] .Polycythemia vera [PV] 11 cases [7.91%] and 10 cases [7.19%] of Primary myelofibrosis [PMF]. [Table 4]

Table 4: Entity wise distribution of cases

Myeloproliferative neoplasms	Total [n=138]	%
Chronic myeloid leukemia	105	76.1
Polycythemia vera	13	9.42
Essential thrombocythemia	10	7.25
Primary myelofibrosis	10	7.25
Total	138	100

Distribution according to presenting complaints

Majority of the cases presented with generalized weakness in 92 [66.7%], followed by malaise and fatigue 57 cases [41.30%], weight loss 51 [37.00%], fever 37 [26.80%]. In addition, 14 [10.10%] patients had abdominal pain, which was attributed to moderate to massive splenomegaly. [Table 5]

Table 5: Distribution according to common presenting complaints

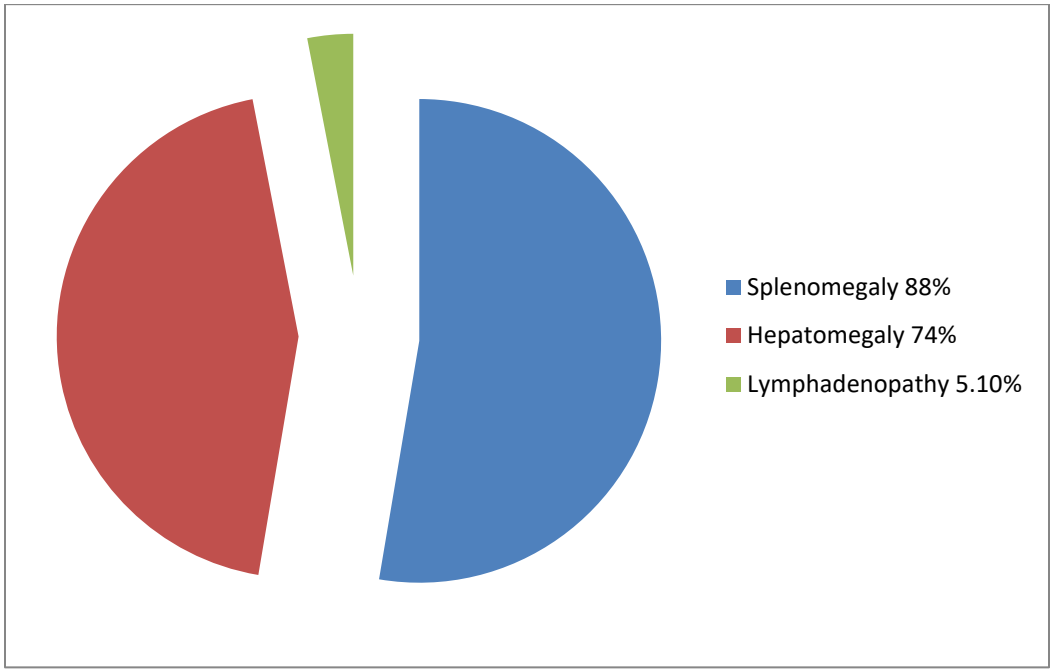
Presenting complaints	No. of cases	%
Generalized weakness	92	66.7
Malaise and fatigue	57	41.30
Weight loss	51	37.00
Fever	37	26.80
Night sweats	12	8.70
Abdominal pain	14	10.10
Headaches	8	5.80
Vertigo	6	4.35
Bleeding tendencies	5	3.62
Dizziness	4	2.90
Paresthesia	3	2.17
Bluish discoloration of digits	3	2.17

Note- many patients had more than 1 complaints at the time of presentation.

Distribution according to organomegaly

Splenomegaly was noted in 121 cases [88.00%], hepatomegaly was noted in 102 cases [74.00%], lymphadenopathy was seen in 7 cases [5.10%].[Figure 2]

Fig 2: Distribution according to presence of organomegaly



Of total 138 cases, 105 [74.64%] on mutational analysis had BCR-ABL positive and were categorized as chronic myeloid leukemia after correlating with hemograms, bone marrow aspiration and biopsy findings and clinical features. Of 105 cases with BCR-ABL fusion gene, 22 had presence of both 210kD and 190kD fusion protein [major and minor transcript], while 83 had only 210kD [major transcript].

CHRONIC MYELOID LEUKEMIA

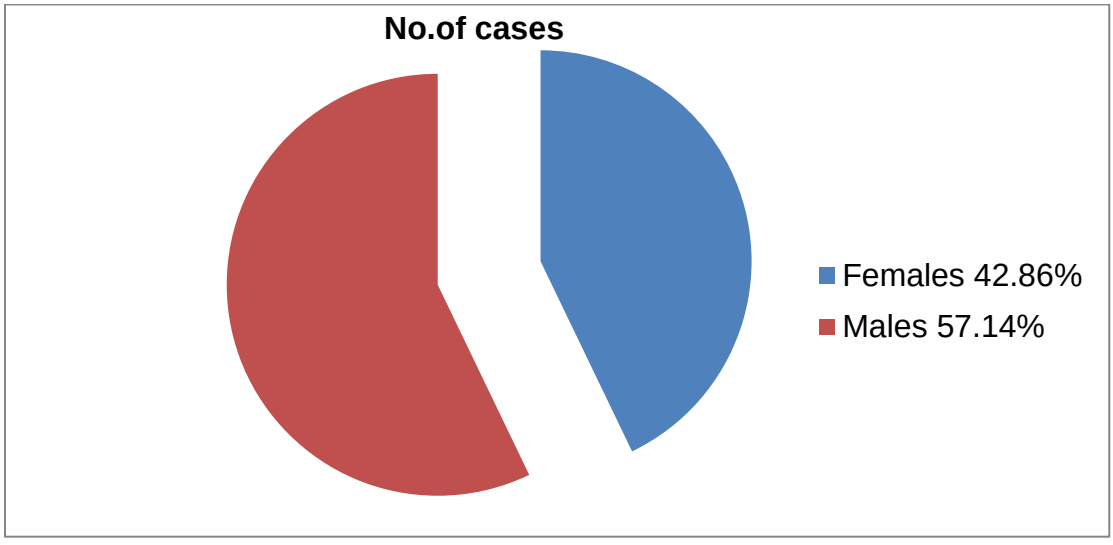
Age and Gender wise distribution.

Of 105 cases of CML which were diagnosed on cytogenetics and hematological profile, majority of patients were in age group of 41-50 years [27 cases 25.70%] followed by 31 to 40 years [20 cases 19.00%] of age group with mean age being 53 years. The youngest patient was 7 years old and the eldest patient was 72 years of age [Table 6]. Slight male predominance was noted with M:F 1.3:3.[males were 60, females were 45 in number] [Figure 3]

Table 6: Distribution according to age

Age [years]	No. of cases	%
< 10	1	0.95
11-20	4	3.81
21-30	19	18.1
31-40	20	19.00
41-50	27	25.70
51-60	17	16.20
61-70	14	13.30
> 70	3	2.86

Figure 3: Distribution of cases according to gender



Technique for confirmation of BCR-ABL positivity

All the 105 cases were BCR-ABL fusion gene positive patients [76.19%] were diagnosed by RT-PCR quantitative, 13 [12.38%] by FISH, 10 [9.52%] by RT-PCR qualitative and 2 cases [1.91%] by NGS panel. [Table 7]

Table 7: Distribution according to technique for confirmation of BCR-ABL

Technique	No. of cases	%
NGS	2	1.91
RT-PCR qualitative	10	9.52
FISH	13	12.38
RT-PCR quantitative	80	76.19
Total	105	100.00%

Clinicalpresentation of patients with CML

Of the 105 patients majority of the patients presented with generalized weakness [71,67.6%] followed by malaise/ fatigue [49,46.7%], weight loss [36,34.3%] ,fever[29, 27.6%] ,and night sweats [10, 9.52%]. Only 6 [5.71%] patients complaints of abdominal pain, which was due to moderate to massive splenomegaly and three [2.86%] patients complaint of bruises, petechiae and purpura, all of these patients had peripheral thrombocytopenia. [Table 8]

Table 8: Distribution of clinical presentations of patients with CML

Presenting complaints	No. of cases	%
Generalized weakness	71	67.6
Malaise and fatigue	49	46.7
Weight loss	36	34.3
Fever	29	27.6
Night sweats	10	9.52
Abdominal pain	6	5.71
Bleeding tendencies*	3	2.86

*These patients had peripheral thrombocytopenia

CML patients presenting with organomegaly and the degree of splenomegaly

Of the 105 cases, [92, 87.61%] had splenomegaly and majority of patients had moderate [52, 49.50%] splenomegaly while moderate to massive [14,13.30%] splenomegaly was noted in 14 [13.3%] cases. In addition, 79 [75%] had hepatomegaly while 5 [4.80%] had lymphadenopathy [of these 5 cases, 4 had past history of tuberculosis]. [Table 9]

Table 9: Distribution of cases according to presence of organomegalyand degree of splenomegaly.

Organomegaly	No.of cases[%]	
Splenomegaly [n=92,87.61%]	Mild [1-3 cm below costal margin]	27 [25.70%]
	Moderate [4-8 cm below costal margin]	52 [49.50%]
	Severe [>8cm or beyond umbilicus]	14 [13.30%]
Hepatomegaly	79 [75.00%]	
Lymphadenopathy	5 [4.80%]	

Hematological parameters of all CML patients [n=105]

Of all 105 CML cases, though 21.9% [n=23] patients had normal hemoglobin levels but majority of patients [54, 50.49%] did present with moderate degree of anemia [Hb 8.1-10.9 gm/dl], 11 [10.68%] patients had severe anemia and 17 [16.50%] patients had mild anemia. On smear examination majority of these had normocytic type anemia [n= 70, 66.66%]

On analyzing total leucocyte count it was observed that majority [98.1%] of patients had varying degree of leucocytosis at presentation with 58 [55.2%] cases having hyperleucocytosis [>100.1X10^3/ul] followedby 23 [21.90%] patients having mild leukocytosis [11.1 to 49.9x10^3/ul] while moderate leucocytosis being seen in 22 cases [21.00%]. Leucocytosis did

show a positive correlation with splenomegaly as 78/80 patients with moderate to marked leucocytosis had significant splenomegaly. On differential leucocyte counts significant left shift in granulocytes was noted in 102 [99.03%]. Other notable features included basophilia [<19%, ranging 1 – 7%] in 63 cases [61.17%] of patients and eosinophilia [<1.5x10^3/ul] in 17 [16.50%] patients.

Blast count ranged from 1 to 38% with only one patient presenting with blast [>20%], rest all the patients had blasts count <10% [ranging 1 to 9%]

Majority patients [75 cases, 71.4%] had normal range platelet count between 100x10^3/ul to 450x10^3/uL. Two patients had thrombocytopenia with platelet count of 95x10^3/ul and 32x10^3/ulwhile 26 patients [24.8%] had thrombocytosis with platelet count between 450 to 1000x10^3/uL.

Bone marrow aspiration was examined for cellularity and maturation of lineages which was confirmed on bone marrow biopsy examination. The megakaryocyte morphology and clustering was analyzed on bone marrow biopsy

Age adjusted hypercellularity with myeloid hyperplasia was seen in all of the cases of CML with mean M:E 8:1. Megakaryocytes were increased in 95 out of 105 cases, [16 had hyperlobated and 58 had dwarf forms]. 58 patients [55.24%] had increased megakaryocytes with presence of dwarf megakaryocytes. 21 [20.00%] had increased megakaryocytes with focal clustering of megakaryocytes, all of these patients had bone marrow fibrosis of grade2 to grade 3 on reticulin stain. 52 out of 105 patients [48.54%] had no increase in reticulin fibrosis on bone marrow biopsy examination.While patients with increased fibrosis was as follow with grade 1 fibrosis [32, 31.07%]. Grade 2 fibrosis [19, 18.45%] and grade 3 fibrosis [2, 1.94%]. Patients with grade2 and 3 fibrosis had TLC ranging from 59 to 394.6x10^3/ul, platelet count ranging from 171 to 918x10^3/ul, Hb ranging from 7.4 to 12.9g/dl with megakaryocytic hyperplasia and focal clustering.

On basis of leukocytosis, thrombocytosis, thrombocytopenia, basophilia and blast count CML cases were further subclassified into 3 phases:

Of 105, 103 cases were in CML Chronic phase with peripheral leukocytosis, basophilia [<20%], blast count <10% and marrow hypercellularity with myeloid surge.

Two cases were categorized into CML accelarated phase. One patient had borderline low TLC-4x10^3/ul and other was a k/c/o CML with mild leukocytosis- 31.5x10^3/ul. Both had thrombocytopenia [32x10^3/ul and 95x10^3/ul respectively] and blast count of 10% and 12% respecytively. [Table 10, 11]

One patient was of CML in Blast crisis with 38% blasts in PBF and 40% on BMB.

Table 10: Distribution according to hematological parameters

Hemoglobin	**Severity of anemia**	**No.of patients**	**%**
	< 8 [severe]	11	10.68
	8.1-10.9 [moderate]	54	50.49
	11-11.9 [mild]	17	16.50
	12-15.9	23	
Total leucocyte count x10^3/ul	4.1-11.0	2	1.90
	11.1-49.9 [mild]	23	21.90
	50-100.0 [moderate]	22	21.00
	> 100.1 [severe]	58	55.20
Differential leucocyte count	Promyelocyte/myelocyte/ metamyelocyte	102	99.03
	Basophilia	63	61.17
	eosinophilia	17	16.50
	Blasts>20%*	01	0.97
Platelet count [normal 150-450x10^3/ul]	< 100.	3	2.86
	100-450	75	71.4
	450-1000	26	24.8
	> 1000	1	0.95

Table 11: Bone marrow aspiration cellularity and erythropoiesis, myelopoiesis and megakaryopoiesis.

	Cellularity	**No.of cases**	**%**
Bone marrow aspiration and biopsy examination	Hypercellular	105	100.00%
	Normocellular	0	00%
	Myelopoiesis		
	Hyperplasia	105	100%
	Megakaryopoiesis		
	adequate	10	9.52
	hyperplasia with focal clustering	21	20.00
	hyperplasia with hyperlobated forms	16	15.24
	hyperplasia with dwarf forms	58	55.24
	Bone marrow biopsy fibrosis on reticulin stain		
	No fibrosis	52	48.54
	Grade1	32	31.07
	Grade2	19	18.45
	Grade3	2	1.94

JAK2V617F POSITIVE

33 cases were BCR ABL, CALR, MPL negative but JAK2v617f mutation positive. These cases were then categorized into ET, PV, PMF aftercorrelatingwith hematological parameters and bone marrow findings.

Essential thrombocythemia

All the 10 cases were confirmed by quantitative RT-PCR. Out of 10, 2 patients had additional BCR-ABL positivity. These 2 patient presented with marked peripheral thrombocytosis and had indeterminate BCR-ABL positive [copy number <10] .correlating with cytogenetics, hematological profile and bone marrow parameters these cases were categorized as essential thrombocythemia.

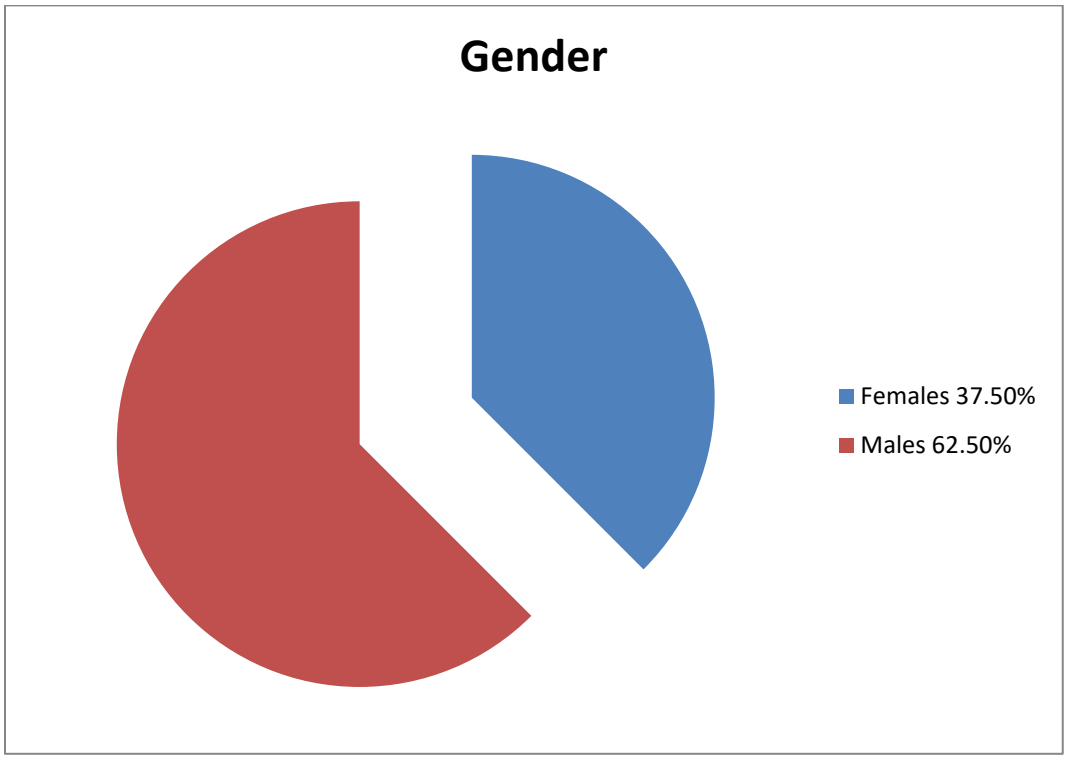
Distribution according to age

All the patients with ET were at or beyond 4th decade of life with mean age- 46.4 years. The youngest was 36 years and eldest was 81 years [Table 12]. Slight male predominance was noted with M:F 1.5:1. [Figure 4]

Table 12: Distribution according to age

Age years	No. of cases	%
< 10	0	00
11-20.0	0	00
21-30	0	00
31-40	02	25
41-50	04	50
51-60	01	12.5
61-70	01	12.5
> 70	00	00
Total	08	100%

Figure 4: Distribution of cases according to gender



Clinical presentation of patients with essential thrombocythemia

Most common complaints were generalized weakness [7, 87.5%], weight loss [5, 62.5%], fever [4, 50%].The unusual presentation was abdominal pain [2, 25%], malaise and fatigue [2,25%], vertigo [2, 25%], headaches [2, 25%], dizziness [1,12.5%], paresthesia [1, 12.5%], bluish discoloration of digits[1, 12.5%].[table13]

Table 13: Depicting the clinical presentations of patients with ET

Presenting complaints	No. of cases	%
Generalized weakness	07	87.5
Weight loss	05	62.5
Fever	04	50
Abdominal pain	02	25
Malaise and fatigue	02	25
Vertigo	02	25
Headaches	02	25
Dizziness	01	12.5
Paresthesia	01	12.5
Bluish discoloration of digits	01	12.5

Patients with organomegaly and the degree of splenomegaly

Splenomegaly was common findings in patients with essential thrombocythemia with Splenomegaly in 9 out of 10 patients [90.0%] with majority [6, 60.0%] having mild splenomegaly. Hepatomegaly was in 6 out of 10 patients [60.0%] [Table 14]

Table 14: Distribution according to presence of organomegaly and degree of splenomegaly

Organomegaly	No.of cases [%]	
Splenomegaly[n=7, 87.50%]	Mild	6 [60.0%]
	Moderate	2 [20.0%]
	Severe	1 [10.0%]
Hepatomegaly	6 [60.0%]	

Hematological parameters of patients with Essential thrombocythemia [n= 10]

All the patients had peripheral thrombocytosis [>450x10^3/uL] with normal range hemoglobin and low to normal hematocrit with mild leukocytosis in 6 out of 10 patients, [60.0%] [TLC ranging 13.2 to 22.3 x10^3/ul]. Bone marrow aspiration was examined for cellularity and maturation and morphology of lineages which was confirmed on bone marrow biopsy examination. Mildly hypercellular marrow was seen in 6 [60%] and 4 [40.0%] had normocellular marrow. There was increase in number of megakaryocytes in all the patients with majority had presence of atypical and hyperlobated nuclei [8 cases, 80%]. Only 2 cases [20%] had clustering of megakaryocytes with increased bone marrow reticulin fibres [Table 15, 16].

Table 15: Distribution of hematological parameters

	No.of cases	%
Platelet count [normal 150-450x10^3/ul]		
< 100.	0	0
100-450	0	0
450-1000	03	30.0
> 1000	07	70.0
Total leucocyte count x10^3/ul		
4.1-11.0	4	40.0
11.1-49.9	6	60.0
50-100.0	0	0
> 100.1	0	0
Differential leucocyte count		
Promyelocyte/myelocyte/metamyelocyte	1	10.0
Basophilia	0	0
eosinophilia	0	0
Blasts>20%*	0	0
Hemoglobin g/dl		
< 8	00	0
8.1-10.9	03	30.0
11-11.9	02	20.0
12-15.9	05	50.0
Hematocrit % [normal range 40-45%]		
< 40	7	70.0
40-48	3	30.0
> 48.1	0	0
< 40	0	0
Red blood cell count [Normal RBC count is 4.5 to 5.5x10^6/ul]		
< 4.4x10^6/ul	7	70.0
4.5-5.5x10^6/ul	3	30.0
>5.6x10^6/ul	0	0
< 4.4x10^6/ul	0	0

Table 16: Bone marrow aspiration cellularity and erythropoiesis, myelopoiesis and megakaryopoiesis.

Bone marrow aspiration and biopsy examination	**Cellularity**	**No.of cases**	**%**
	Hypercellular	6	60.0
	Normocellular	4	40.0
	Erythropoiesis		
	Depressed	1	10.0
	Normal	9	90.0
	Leucopoiesis		
	Orderly cell maturation	9	90.0
	Depressed	1	10.0
	Megakaryopoiesis		
	Adequate	0	00
	Hyperplasia with atypical forms and clustering	2	20.0
	Hyperplasia with atypical forms	8	80.0
	Hyperplasia with dwarf forms	0	00
	Bone marrow biopsy fibrosis on reticulin stain		
	No fibrosis	0	0
	Grade 1	1	10.0
	Grade 2	0	0
	Grade 3	0	0

Polycythemia vera

Out of 33 BCR ABL negative and JAKV617F positive cases 13 were confirmed as polycythemia vera on correlating cytogenetics with hematological profile.

Molecular Techniques for confirmation

10 [76.92%] patients were confirmed with RT-PCR qualitative and 3 [23.08%] were confirmed by RT-PCR quantitative.

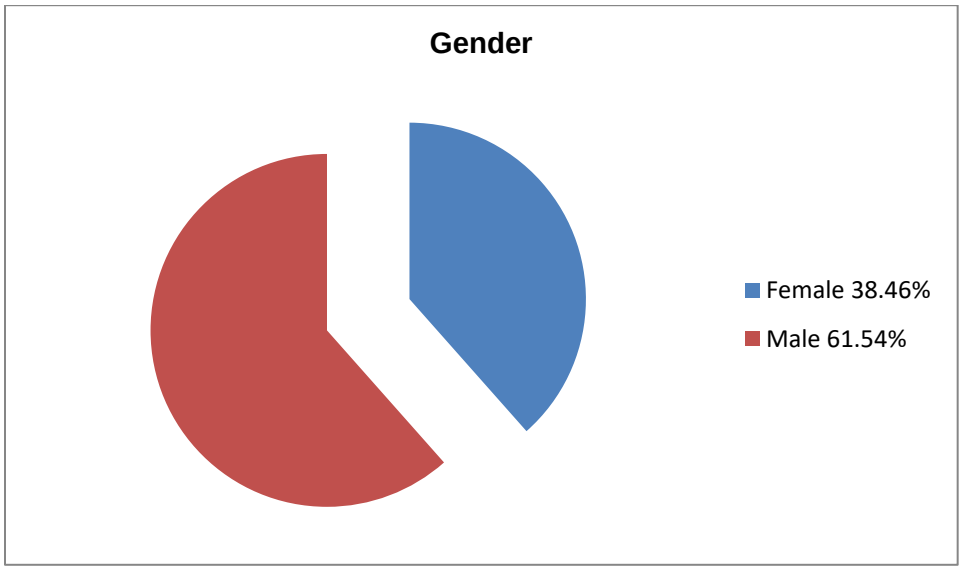
Age and gender distribution of patients with polycythemia vera

Age of these patients ranged from 22 to 76 years old. Majority of patients were between 61-70years. [6, 46.20%]. Mean age- 61 years. Slight male predominance was noted with M:F 1.6:1 [Table 17, Figure 5]

Table 17: Distribution according to age.

Age years	No. of cases	Percentage
< 10	0	0
11-20.0	0	0
21-30	1	7.7
31-40	0	0.0
41-50	3	23.0
51-60	2	15.4
61-70	6	46.2
> 70	1	7.7
Total	13	100

Figure 5: Distribution according to gender



Chief presenting compliants of patients with Polycythemia Vera

Most common complaints were generalized weakness 6 [46.15%] cases, weight loss 6 [46.15 %] , headaches in 5 [38.46%]. Other lesser common complaints were of malaise and fatigue seen in 4 [30.77 %] , vertigo in 4 [30.77%], dizziness in 3 [23.08%] , paresthesia and abdominal pain in 2 [15.38%] each. [Table 18]

Table 18: Distribution according to chief complaints

Presenting complaints	No. of cases	%
Generalized weakness	6	46.15
Weight loss	6	46.15
Headaches	5	38.46
Malaise and fatigue	4	30.77
Vertigo	4	30.77
Dizziness	3	23.08
Paresthesia	2	15.38
Abdominal pain	2	15.4

Patients with organomegaly and degree of splenomegaly

Most of the patients had splenomegaly [11, 84.62 %] with majority having mild splenomegaly [7, 53.85%]. 7 [53.85%] had hepatomegaly and 1 [7.69%] had lymphadenopathy [patient was k/c/o tuberculosis and was on ATT] [Table 19]

Table 19: Distribution according topresence of organomegalyand degree of splenomegaly

Organomegaly	No.of cases[%]	
Splenomegaly [n=11,84.62%]	Mild	5 [62.50%]
	Moderate	2 [25.00%]
	Severe	1 [12.50%]
Hepatomegaly	7 [53.85%]	
Lymphadenopathy	1 [7.69%]	

Hematological parameters of patients with Polycythemia vera [n=13]

Majority patients [10 cases, 76.92%] had thrombocytosis with platelet count ranging from 477 to 1394 x10^3/uL, only 3 [23.08%] patients had normal range platelet count.All the patients had >16 g/dl hemoglobin with raised RBC count [ranging from 6.87x10^6/ulto 9.86 x10^6/uL]and hematocrit [>49%]. 10 [76.92%] patients had mild leukocytosis with TLC ranging from 11.2 to 24.5 x10^3/ul. On bone marrow examination all patients hadtrilineage hyperplasia with mean M:E 1:1. The megakaryocyte morphology and clustering was analyzed on bone marrow biopsy and all the patients had megakaryocytic hyperplasia with atypical megakaryocytes in7 cases [5.84%] and clustering of megakaryocytes in 4 cases [30.77%], of these 4 cases only 2 had bone marrow grade 1 fibrosis. Fibrosis was an uncommon finding in patients with polycythemia vera. [Table 20, 21]

Table 20: Distribution of Hematological parameter

Hematological parameters	No. of cases	%
Total leucocyte count x10^3/ul		
4.1-11.0	3	23.08
11.1-49.9	10	76.92
50-100.0	0	0
> 100.1	0	0
Platelet count [normal 150-450x10^3/ul]		
< 150	0	0
150-450	3	23.08
450-1000	7	53.84
> 1000	3	23.08
Hemoglobin g/dl		
< 8	0	0
8.1-10.9	0	0
11-11.9	0	0
12-15.9	0	0
>16	13	100
Hematocrit % [normal range 40-45%]		
< 40	0	0
40-48	0	0
> 48.1	13	100
Red blood cell count [Normal RBC count is 4.5 to 5.5x10^6/ul]		
< 4.4x10^6/ul	0	0
4.5-5.5x10^6/ul	0	0
>5.6x10^6/ul	13	100

Table 21: Bone marrow aspiration cellularity and erythropoiesis, myelopoiesis and megakaryopoiesis.

Bone marrow aspiration and biopsy examination	**Cellularity**	**No.of cases**	**%**
	Hypercellular	13	100
	Normocellular	0	0
	Erythropoiesis		
	Depressed	0	0
	Normal	4	30.77
	Hyperplasia	7	69.23
	Leucopoiesis		
	Orderly cell maturation	4	30.77
	Depressed	9	69.23
	Megakaryopoiesis		
	adequate	2	15.38
	hyperplasia with atypical forms and clustering	7	53.85
	hyperplasia with atypical forms	4	30.77
	Bone marrow biopsy fibrosis on reticulin stain		
	No fibrosis	0	0
	Grade 1	2	15.38
	Grade 2	0	0
	Grade 3	0	0

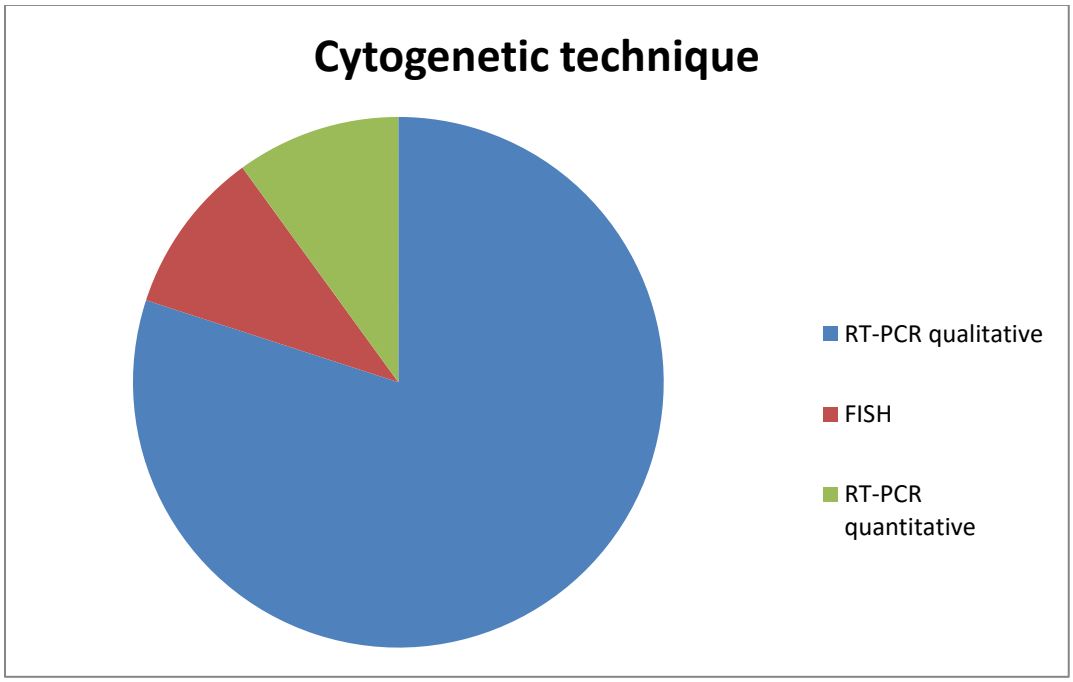
Primary myelofibrosis

10 cases out of 33 BCR ABL negative and JAK2V617f mutation positive cases were classified as Primary myelofibrosis after correlating with hematological parameters.

Technique for confirmation of molecular genetics

8 [80%] cases were confirmed by RT-PCR qualitative and 1 [10%] case each was confirmed by FISH and RT-PCR quantitative. [Figure 6]

Figure 6: Techniques used to confirm the diagnosis



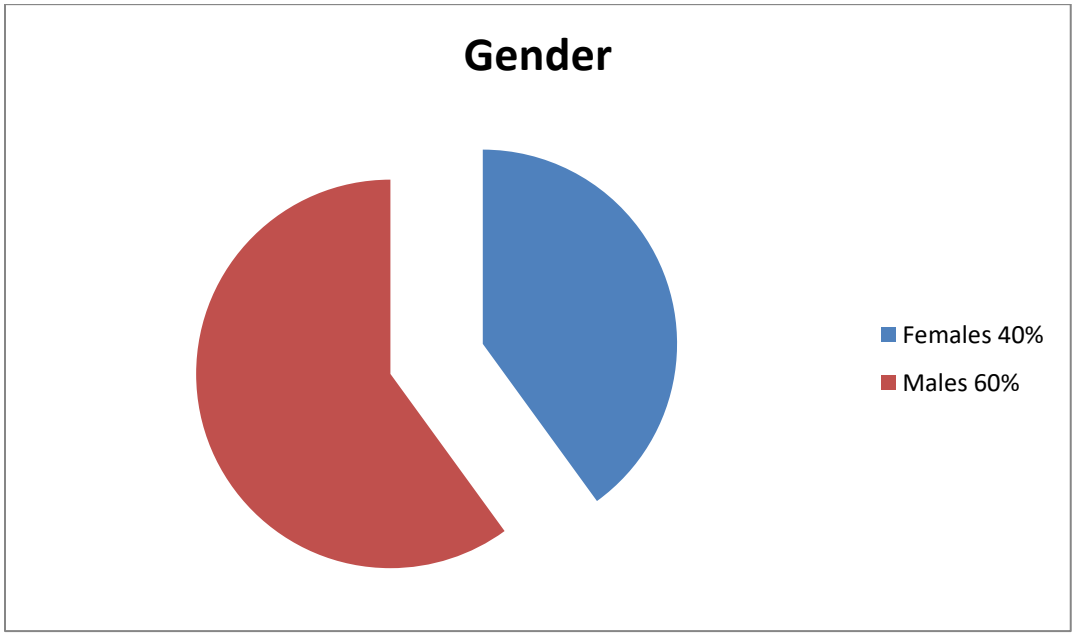
Age and sex distribution of cases with Primary myelofibrosis [n=10]

Primary myelofibrosis was commonly seen in older age group.Age ranged from 46 to 73 years of age with 6 patients [60%] in 61-70 years of age. Slight male predominance was noted with M:F 1.5:1 [Table 22, Figure 7]

Table 22: Distribution according to age

Age years	No. of cases	%
< 10	0	0
11-20.0	0	0
21-30	0	0
31-40	0	0
41-50	1	10
51-60	2	20
61-70	6	60
> 70	1	10
Total	10	100

Figure 7: Distribution according to gender



Clinical presentation of patients with Primary myelofibrosis [n=10]

Most common presenting complaints were generalized weakness 7 [70%], followed by fever 6 [60%] patients, abdominal pain and weight loss seen in 4 [40%] patients respectively with all 4 had organomegaly on physical examination.Lesser common symptoms seen were malaise and fatigue seen in 2 [20%] patients, night sweats was seen in 2 [20%] patients. [Table 23]

Table 23: Distribution according to presenting complaints

Presenting complaints	No. of cases	%
Generalized weakness	7	70
Malaise and fatigue	2	20
Weight loss	4	40
Fever	6	60
Night sweats	2	20
Abdominal pain	4	40

Note- each patient presented with more than one complaints.

PMF patients with organomegaly and degree of splenomegaly

All the patients of Primary myelofibrosis had organomegaly. Splenomegaly was noted in 8 [80%] with majority had moderate to massive splenomegaly [7, 70%], while hepatomegaly in 8 [80%] and 1[10%] patient had lymphadenopathy. [Table 24]

Table 24: Distribution according to organomegaly and degree of splenomegaly

Organomegaly	No.of cases[%]	
Splenomegaly [n=8,80%]	Mild	1 [10%]
	Moderate	4 [40%]
	Severe	3 [0%]
Hepatomegaly	8 [80%]	
Lymphadenopathy	1 [10%]	

Hematological parameters of patients with Primary myelofibrosis

Six [60%] patients had normal range TLC ranging 4.6 to 10.4 x10^3/uL], 3 [30%] had leukocytosis TLC ranging from 23.8 to 135x10^3/ul, 1 [10%] had mild leucopenia TLC 3x10^3/ul. 4 [40%] patients had shift to left uptomyelocyte stage on PBF.

Seven [70%] had normal platelet count ranging 172 to 404x10^3/uL, thrombocytopenia was noted in 3 [30%] with platelet ranging 80 to 111x10^3/ul.

Majority patients had anemia with 7[70%] hadmoderate anemia ranging from 8.1 to 10.7 g/dl and 2 [20%] had severe anemia with Hb 7.6 and 5.6 g/dl respectively. With majority having microcytic hypochromic anemia with presence of nRBCson PBF. All patients of primary myelofibrosis had low hematocrit ranging 23 to 39% and 7 [70%] patients had reduced RBC count.

Bone marrow aspiration was examined for cellularity, morphology and maturation of lineages which was confirmed on bone marrow biopsy examination. The megakaryocyte morphology and clustering was analyzed on bone marrow biopsy. 5 patients [50%] had hypercellular and 5 patients [50%] had hypocellular marrow. 6 patients [60%] had depressed erythropoiesis with normoblastic maturation, 7 [70%] had depressed myelopoiesis. Megakaryocytic hyperplasia was noted in 7 [70%] with clustering in 5 [50%] cases, 2 cases had depressed megakaryopoiesis. Grade 1 to grade 3 fibrosis was seen in all cases with grade 3 fibrosis in 4 [40%] , grade 2 in 2 [20%] and grade 1 in 4 [40%] [Table 25, 26]

Table 25: Distribution of hematological parameter

Hematological parameters	No. of cases	%
Total leucocyte count x10^3/ul		
4.1-11.0	1	10
11.1-49.9	6	60
50-100.0	2	20
> 100.1	0	0
Platelet count [normal 150-450x10^3/ul]		
< 100.	1	10
100-450	9	90
450-1000	0	0
> 1000	0	0
Hemoglobin g/dl		
< 8	2	20
8.1-10.9	7	0
11-11.9	0	0
12-15.9	1	10
Hematocrit % [normal range 40-45%]		
< 40	10	100
40-48	0	0
> 48.1	0	0
Red blood cell count [Normal RBC count is 4.5 to 5.5x10^6/ul]		
< 4.4x10^6/ul	7	70
4.5-5.5x10^6/ul	3	30
>5.6x10^6/ul	0	0

Table 26: Bone marrow aspiration cellularity and erythropoiesis, myelopoiesis and megakaryopoiesis.

Bone marrow aspiration and biopsy examination	**Cellularity**	**No.of cases**	**%**
	Hypercellular	5	50
	Normocellular	5	50
	Erythropoiesis		
	Depressed	6	60
	Normal	4	40
	Hyperplasia	0	0
	Leucopoiesis		
	Hyperplasia	1	10
	Orderly cell maturation	2	20
	Depressed	7	70
	Megakaryopoiesis		
	Hyperplasia with clustering	7	70
	Depressed	2	20
	Adequate	1	10
	Bone marrow biopsy fibrosis on reticulin stain		
	No fibrosis	0	0
	Grade 1	4	40
	Grade 2	2	20
	Grade 3	4	40

DISCUSSION

The present five year study [3.5 years retrospective;1st April 2016– 30th September 2019 and 1.5 years prospective;1st October 2019 – 31st March 2021] was conducted in the Department of Pathology, Dayanand Medical College and Hospital, Ludhiana. During this study a total of 138 confirmed myeloproliferative neoplasms [CML, ET, PV, PMF] and were analyzed with respect to genetic profile, clinical findings, hematological parameters and bone marrow features. Of 138 cases included majority [n=35, 25.4%] were in the age group of 41 to 50years of age group with age range of 7 to 81 years with mean age being 47 years. Slight male predominance was noted with M:F ratio of 1.3:1. This was found be in concordance with studies conducted by Yassin et.al [86] and Yap et.al [87] and Kumar et.al [88] [Table 27]

Table 27: Comparison of age and sex with other studies

Authors	Age range	Common age group affected	Mean age	M:F
Kumar et.al[88]	5-72 years	31-40years	38.6 years	1.4:1
Yap et.al[87]	25-70 years	50-60	54.2	1.2:1
Yassin et.al[86]	18-80 years	55-65	58	1:1
Present study	7-81 years	41-50	47	1.3:1

It was observed that majority of the patients had generalized weakness [n=92, 66.7%], followed by malaise/ fatigue in [n= 57, 41.3%], weight loss [n= 51, 37.0%], and fever [n=37, 26.8%]. Our observations are concordant with Kumar et.al.[88], who concluded that weakness [63 %], fever [59%], fatigue [55.5%], and weight loss [54.4 %] were common symptoms of patients with MPNs. Similarly, constitutional symptoms such as weakness, exhaustion, and fever were also observed in 60% of cases in a study conducted by Ganesan et.al.[89] However, our findings slightly differed with those of Anderson et.al.[90] who reported that fatigue as

common complaint in 74% of cases, while weight loss [6.5%] and fever [4.9%] were less common.

Furthermore, 14 [10.10%] of the patients in our study experienced abdominal pain, which can be attributed to moderate to severe splenomegaly, comparable to study done by Yap et.al[87], which found that 32.2 % of patients who presented with abdominal pain had a palpable spleen on examination. Overall, in our cohort splenomegaly was observed in 121 [88.0%], hepatomegaly in 102 cases [74.00 %], and lymphadenopathy in 7 cases [5.10 %] [5 out of 7 cases had a history of tuberculosis]. This observationsare concordant with Kumar et al. [88], who reported splenomegaly in 62.2 % of patients, hepatomegaly in 57.7 %, and lymphadenopathy in 6%.

Chronic Myeloid Leukemia

Of the 138 cases of myeloproliferative neoplasms, majority [n=105, 76.08 %] were classified as chronic myeloid leukemia according to WHO criteria [2016]. After correlating the hematological parameters and bone marrow findings, of these 105 cases of CML, 102 cases [97.14%] were categorized into chronic phase while 2 cases [1.90%] were observed to be in accelerated phase and one case [0.95%] was in blast crisis. Similarly, in a study by Bansal et al. [91], 97 % of cases were in the chronic phase of CML, whereas the remaining 3 % were in the accelerated phase.

Similarly, though Bhatti et al[75], also observed CML being the predominant [335/425] MPN and majority being in chronic phase 240 cases [71.6 %] but accelerated phase [n= 49,14.6 %] and blast phase [n= 31 cases,9.3 %] category patients were significantly higher as compared to our observation. This could be attributed to larger sample size in their study

Gene transcript expression

Among the 105 cases, 83 [79.04 %] had only 210kD, while 22 [20.95 %] showed co-expression of 210kD and 190kD. This is consistent with the

findings of Trejo et al. [92], who found that 226/250 patients with CML had p210kD alone, whereas the remaining 9.6 % had co-expression of the transcripts p190kD and 210kD. Similarly, Yaghmaie et.al [93] discovered that 83 % of patients expressed only p210kD, while the remaining 17 % expressed both 210kD and 190kD.

Age and sex distribution

We observed that majority of CML patients [27 cases, 25.70 %] were in the middle age group 41-50 years [27 cases, 25.70 %], followed by 31 to 40 years [20 cases, 19 %] with a mean age of 53 years, and M:F ratio of 1.3:1. Kumar et al. [88], observed the most common age group affected was between 31 and 40 years old, with a mean age of 38.6 years, with M:F ratio of 1.4:1 [57.8 % males and 42.2 % females]. Singhal et al. [94], reported the mean age of presentation in CML patients was 55 years, with a slight male predominance and a M:F ratio of 1:0.8. Similarly, in a study conducted by Ganesan et al. [89], the average age of presentation was 47 years, with a M:F ratio of 1.6:1. These studies coincided with our findings. Similarly, the findings of Mottalib et al [95], Bansal et al [91], Savage et al [96], and Hoffmann et al [97] are consistent with our findings. [Table 28]

Table 28: Comparison of age and sex distribution with other studies

Authors	Mean age [years]	M:F
Kumar et.al[88]	38.6	1.4:1
Singhal et.al[94]	55	1:0.8
Ganesha et.al[89]	47	1.6:1
Mottalib et.al[95]	37.4	2:1
Bansal et.al[91]	42	3:1
Savage et.al[96]	34	1.3:1
Hoffmann et.al[97]	56	1.2:1
Present study	53	1.3:1

Clinical presentations and organomegaly

The majority of patients in our study had generalized weakness [71, 67.6 %], followed by malaise/ fatigue [49, 46.7 %], weight loss [36, 34.3 %], fever [29, 27.6 %], and night sweats [10, 9.52 %]. Less prevalent complaints included abdominal pain [6, 5.71 %] attributed to moderate to severe splenomegaly. Three [2.86 %] patients presented with bruises, petechiae, and purpura, and all of them had peripheral thrombocytopenia.

These findings are consistent with those of Kumar et al. [88], they observed that the most prevalent complaints of patients were generalised weakness [63 %], fever [59 %], and fatigue [55.5 %]. They reported that 99 % of the patients had splenomegaly, which is consistent with our findings. In our study we observed splenomegaly in 92/105 cases [87.61 %]. Kumar et al. [88] likewise reported that splenomegaly was the most common symptom, accounting for the majority of cases. Massive splenomegaly was present in 42 [56%] of the patients. 13 [17.3%] patients exhibited mild splenomegaly, whereas 20 [26.6%] cases had moderate splenomegaly. This conclusion is in contrast to our study, we found that majority of cases had moderate [52, 49.50 %] splenomegaly, while 27 patients25.7%] had mild splenomegaly and 14 patients [13.33%] had massive splenomegaly. Singhal et al. [94] reported splenomegaly in up to 100% of patients. However, in contradiction Cotta CV et al [98] reported that 50% of CML patients showed splenomegaly.

According to a studies conducted by Ganesan et al [89], Bansal et al [91], and Hoffmann et.al [97], all reported that a higher proportion of patients have organomegaly and commonly presented with non-constitutional symptoms. [Table 29]

Table 29: Frequency of splenomegaly in CML patients.

Authors	% of CML patients with splenomegaly
Kumar et.al[88]	83.33%
Singhal et.al[94]	100%
Ganesan et.al[89]	95%
Bansal et.al[91]	80%
Cotta CV et.al[98]	50%
Hoffmann et.al[97]	46.5%
Present study	87.61%

Hematological parameters

We observed that majority of CML patients [54, 50.49 %] presented with moderate anemia [Hb 8.1-10.9 gm/dl], 11 [10.68 %] had severe anemia, and 17 [16.50 %] had mild anemia. Similar observations were made by Kumar et al. [88] with 45 [64.21 %] of the cases having moderate anemia while mild anemia, and severe anemia being noted in 30% and 5.7% respectively. Further, Singhal et al [94], Mottalib et al[95], Bansal et al[91] and Hernadez et al[99] also reported concordant findings [Table 30] However, Jonte et al. [100] observed most patients with mild anemia [47.4 %], while moderate anemia [36%], and severe anemia [16.6 %] were less frequent.

Table 30: Distribution of hemoglobin

Authors	**% of patients with moderate anemia**	**% of patients with mild anemia**	**% of patients with severe anemia**
Kumar et.al [88]	64.21%	30%	5.71%
Singhal et.al [94]	78%	14%	10%
Mottalib et.al [95]	58.33%	33.33%	10.41%
Bansal et.al [91]	60%	60%	15%
Present study	50.49%	50.49%	10.68%

In our study majority [98.1 %] of patients had varying degrees of leucocytosis at presentation, with 58 [55.2 %] cases having hyperleucocytosis [>100.1X103/ul], 22 cases [21 %] having moderate leucocytosis [TLC ranging 50-100x103/uL], and 23 cases [21.90 %] having mild leucocytosis [TLC ranging 11.1/49.9x103/uL]. This is concordant with the findings of Kumar et al. [88], they concluded that the majority of cases [77.8 %] had hyperleukocytosis, 20.2 % had moderate leukocytosis, and 2% had mild leukocytosis. Similar conclusions were reached in studies conducted by Mottalib et al [95], Bansal et al [91], Singhal et al [94], and Hoffmann et al.[97][Table 31]

Table 31: Distribution of hyperleucocytosis

Authors	TLC <50x10^3/ul	TLC 50-100x10^3/ul	TLC >100x10^3/ul
Kumar et.al [88]	2%	20.2%	77.8%
Mottalib et.al [95]	6.25%	33.33%	72%
Bansal et.al [91]	6.5%	26.5%	67%
Singhal et.al [94]	7.7%	30%	62.3%
Hoffmann et.al [97]	12%	20.7%	67.3%
Present study	21.90%	21%	58.2%

In our study, we observed 78/80 [97.5 %] of patients with moderate to severe leukocytosis had significant splenomegaly. Similarly Abramson et al. [101] reported that patients with severe leucocytosis had splenomegaly.

A significant left shift in granulocytes was seen in 102 patients [99.03 %] when differential leucocyte counts were examined. Basophilia [range from 1 to 7 %] was observed in 63 cases [61.17 %], while eosinophilia was observed in 17 cases [16.50 %]. Similarly, in a study conducted by Kumar et al. [88], basophilia was found in 98.8 % of cases while eosinophilia was found in 20% of cases. Similar observations were made by Jonte et al [100] and Hoffmann et al.[97] [Table 32]

Table 32: Differential leucocyte count

Authors	% of cases with basophilia	% of cases with eosinophilia
Kumar et.al [88]	98.8%	20%
Jonte et.al [100]	83%	78%
Hoffmann et.al [97]	90%	30%
Present study	61.17%	16.50%

Majority of patients in our study, 97.1% had blast count < 10%. Only one patient [0.95 %] in our study presented with blast crisis, with a blast count of 40%. This patient had moderate leucocytosis [54.9x103/ul], mild thrombocytosis [550x103/uL], severe anaemia [6.5g/dl] and grade 1 fibrosis on bone marrow biopsy. Similarly, Kumar et al. [88] reported that just 5% of cases, Hoffmann et al. [97] reported that 2.2 % of cases, and Mottalib et al. [95] reported that 3% of cases were in a blast crisis. This could be attributed to larger sample size in their study. In another investigation, Jonte et al [100] observed that none of the patients were in a blast crisis.

Two of the patients had blast counts of 10% and 12%, respectively, with thrombocytopenia [platelet counts of 32x103/ul and 95x103/ul respectively] that was unresponsive to treatment. On reticulin staining done on bone marrow biopsy, both of these patients had grade 1 fibrosis. These patients were diagnosed with CML at the accelerated phase.

Bhatti et al. [75] reported that majority of CML patients [n = 240, 71.6 %] were in the chronic phase of the disease, with 49 [14.6 %] in the accelerated phase and 31 [9.3 %] in the blast crisis. , which is consistent with our observations that 103/105 [98%] cases were in the chronic phase, 2 [1.95 %] patients being in the accelerated phase, and 1 [0.95 %] patient was in blast crisis. Similarly, Mottalib et al. [95] reported that out of a total of 62 cases, the majority [86 %] were classified as chronic phase, 11% as accelerated phase, and 3% as blast crisis. Hoffmann et al. [97] also

observed that 94.3 % cases were in the Chronic phase, 3.5 % were in the accelerated phase, and 2.2 % were in the Blast Crisis. Similar findings were reported by Kumar et al. [88], they observed that 75/90 cases [83 %] were in chronic phase of CML, 11 patients [12%] were in accelerated phase, and 4 patients [5%] were in blast crisis phase.

In the present study, the majority of patients [77 cases, 71.4 %] had platelet counts in the normal range, ranging from 100x103/ul to 450x103/uL. Two patients had thrombocytopenia with platelet counts of 95x103/ul and 32x103/ul [these patients had blast counts of 10% and 12%, respectively, and were categorized as CML in accelerated phase], while 26 patients [24.8%] had thrombocytosis with platelet counts ranging from 450 to 1000x103/uL. Similarly Savage et al. [96] also reported that the majority of patients [47 %, 155 cases out of 430] had normal platelet counts, 36.2 % had thrombocytosis, and just 4.5 % had thrombocytopenia. Similar observations were made in studies conducted by Mottalib et al. [95], Kumar et al. [88]. [Table 33]

These findings were contradicted by Jonte et al. [100] they reported that the majority of patients [48 %] had thrombocytosis, 41 % had normal platelet counts, and 11 % had thrombocytopenia. [Table 33]

Table 33: Distribution of platelet counts

Authors	Normal range platelet count [150-450x10^3/ul	Thrombocytopenia <150x10^3/ul	Thrombocytosis >450x10^3/ul
Savage et.al[96]	7%	4.5%	36.2
Mottalib et.al[95]	81.2%	8.4%	10.4%
Kumar et.al[88]	68.9%	6.7%	24.4%
Jonte et.al[100]	41%	11%	48%
Present study	71.4%	1.94%	24.8%

Bone marrow aspiration and biopsy findings

On bone marrow aspiration and biopsy examination, age-adjusted hypercellularity was found in all 105 cases in our study. Myeloid hyperplasia was also observed in all of these cases, with a mean M:E ratio of 8:1. Megakaryocytes were also increased in 95/105 cases, with hyperlobated forms seen in 16 cases [16.84%], dwarf forms seen in 58 cases [55.24%], and focal clustering of megakaryocytes seen in 21 cases [20%]. On reticulin stain, all 21 cases [20%] with megakaryocytic clustering had increased bone marrow biopsy fibrosis of grade 1 to grade 3. Similarly, Khonglah et al. [102] concluded that all 55 cases [100%] had hypercellular marrow with myeloid hyperplasia. Mean M:E ratio was 10:1. Megkaryocytic hyperplasia was observed in 71% of the cases [dwarf forms in 85%, and hyperlobated forms in 18% of the cases]. Increase in bone marrow biopsy fibrosis was seen in 73 % of cases.

Thiele et al [103], Cervantes et al [104], and Jonte et al [100] all reported similar observations [Table 34]

Table 34: Bone marrow aspiration and biopsy findings

Authors	% of patients with hypercellularity	Mean M:E	Blasts %	Megakaryocyte morphology	Fibrosis grade
Khonglah et.al [102]	100%	10:1	1-10%	Dwarf forms-85% Hyperlobated forms-18%	73%
Cervantes et.al [104]	100%	8:1	1-16%	Dwarf forms-86% Hyperlobated forms-14%	100%
Thiele et.al [93]	100%	12:1	1-27%	Dwarf forms-96% Hyperlobated forms-4%	84%
Jonte et.al [100]	100%	9:1	1-58%	Dwarf forms-92.4% Hyperlobated forms-7.6%	41%
Present study	100%	8:1	1-40%	Dwarf forms-59.8% Hyperlobated forms- 16.84%	20%

JAK2V617F positive

On cytogenetic mutational analysis, 33 cases out of 138 were BCR ABL negative but JAK2v617f positive. Of these 33, 7.25% [10 cases] had marked peripheral thrombocytosis with normal range hemoglobin, RBC count and hematocrit. These cases were classified as ET based on the clinical presentation, hemogram, bone marrow biopsy, and aspiration findings. Of these 10 cases grade 1 fibrosis was seen in 2 patients on bone marrow biopsy.

Of these 33 cases, 39.4% [13 cases] had elevated haemoglobin [>16g/dl], RBC count [>6x10^6/uL], and hematocrit [>49 %].Thrombocytosis was noted in 76.92% [10/13 cases]. On bone marrow aspiration and biopsy examination panmyelosis which was seen hyperplasia of myeloid, erythroid and megakaryocytic lineages was observed in all 13 cases. These cases were classified as PV. Of these 13 cases, 2/13, 15.4% cases had grade 1 bone marrow fibrosis.

Ten of the 33 cases had normal TLC and platelet counts, with moderate anemia in 7/10 [70%] of the cases, as well as tear drop cells and a leucoerythroblastic peripheral blood picture. Megakaryocytic hyperplasia [7/10 cases] and megakaryocytic clustering [5/10 cases] were found during bone marrow aspiration and biopsy examination. All ten cases had fibrosis ranging from grade 1 to grade 3. Thus, these cases were classified as PMF based on their clinical manifestations, hemogram, bone marrow aspiration, and biopsy findings.

Essential thrombocythemia

Age and gender distribution

The patients with ET were all in their fourth decade of life or older, with a mean age of 46.4 years. The youngest was 36 years old, while the oldest was 81. A slight male predominance was noted with M:F ratio of 1.5:1. A study done by Gisslinger et.al.[105] observed 259 individuals with a mean

age of 57.4 years and an age range of 18-88 years. However, gender distribution was discordant with M:F 0.6:1in Gisslinger et.al[105] study. Fenaux et al [106], Hehlmann et al [107], Thiele et al [108], and Rumi et al [109] all reported similar findings. [Table 35]

Table 35: Mean age and M:F

Authors	Mean age [years]	M:F
Gisslinger et.al [105]	57.4	0.6:1
Fenaux et.al [106]	60	1:1.3
Hehlmann et.al [107]	58	1.1:1
Thiele et.al [108]	67	1:1
Rumi et.al [109]	54	1:1.5
Present study	46.4	1.5:1

Clinical presentations and organomegaly

Generalized weakness [7, 87.5 %], weight loss [5, 62.5 %], and fever [4, 50 %] were the most prevalent complaints in our study. Abdominal discomfort [2, 25%], malaise and fatigue [2, 25%], vertigo [2, 25%], headaches [2, 25%], dizziness [1,12.5%], paresthesia [1, 12.5 %], bluish staining of digits [1, 12.5 %] were all unusual symptoms. Similarly, the most prevalent presenting symptoms in a study by Gisslinger et al. [101] were constitutional complaints such as generalized weakness [80.2 %], malaise and fatigue [77.22 %], and weight loss [8.6 %]. Another study by Thiele et al. [104] found that generalized weakness [86 %], malaise and fatigue [78.4 %] were more common presenting complaints while weight loss in 30 cases [14.42 %], and fever in 25 cases [89.28 %] were unusual presenting complaints.

In the current study, 50% of the patients had complaints relating to thromboembolic events, which was in contrast to Hehlmann et.al [107] study, in which 84 % of the participants had thromboembolic problems.

Our findings are similar to those of Fenaux et.al[106], who found thromboembolic events in 50 of 147 patients [34.01%].

Splenomegaly was also a common finding in patients with essential thrombocythemia in our study, with the majority of patients [6, 60.0 %] had mild splenomegaly.This finding contrasted with that of Gisslinger et al. [105], who found that only 16.7% of patients had splenomegaly, and Barbui T et al. [110], who found that only 20% of patients had splenomegaly.

The findings in our study are concordant to those of Hehlmann et al [107], who found splenomegaly in 42 % of cases, and Thiele et al [108], who found splenomegaly in 52 % of cases [Table 36]

Table 36: Distribution of splenomegaly

Authors	% of cases with splenomegaly
Barbui T et.al[110]	20%
Thiele et.al[108]	52%
Hehlmann et.al[107]	42%
Gisslinger et.al[105]	16.7%
Present study	90%

Hematological parameters

All of the patients in our study had hemoglobin, RBC count, and hematocrit levels that were within normal limits. These results correspond with those of Gisslinger et.al[105], who found that all 259 patients had normal hemoglobin, RBC count, and hematocrit levels. Similarly, Fenaux et al. [106] found that 91 % of cases had normal hemoglobin, hematocrit, and RBC count. Another study conducted by Hehlmann et al [107] found that 92 % of ET cases had normal range hemoglobin. Thiele et al. [108] found that none of the ET cases had elevated hemoglobin levels, [46 % had normal range hemoglobin and 54 % had mild anemia].

Also, in our study, all of the patients [100%] had peripheral thrombocytosis [3 patients, 30% had platelet count of >450-1000x103/uL, 7 patients, 70%

had platelet count of >1000x103/ul], which is consistent with the findings of Gisslinger et al [105] who found peripheral thrombocytosis in all 259 patients [100%]. Fenaux et al. [106] found thrombocytosis in all 147 [100%] of their patients in another study. Similarly, all ET patients in another study conducted by Rumi et al [109] had significant peripheral thrombocytosis.

In our study, 6 out of 10 patients [60.0 %] had mild leukocytosis [TLC ranging from 13.2 to 22.3 x103/ul]. In a study conducted by Gisslinger et al.[105], it was discovered that only 21% of ET patients had mild leukocytosis [TLC ranged from 3 to 31.3x103/uL]. In another study, Fenaux et al [106] found that 66/147 patients [45 %] had mild leukocytosis [TLC ranged from 10 to 29 X 103/ul]. In another study, Thiele et al [108] found that only 9% of ET patients had mild leukocytosis.

Bone marrow aspiration and biopsy findings

In our study, 6 [60%] of the patients had mildly hypercellular marrow, while 4 [40.0 %] had normocellular marrow, with an increase in the number of megakaryocytes in all patients. Atypical and hyperlobated megakaryocytes were seen in 8 cases [80%] and megakaryocyte clustering in 2 cases [20%]. Similarly, Thiele et al. [108] found normocellular bone marrow in 90% of cases, with megakaryocytic hyperplasia in all cases. In another study, Fenaux et al [106] found that bone marrow examination revealed normocellular marrow in 36% of cases and mildly hypercellular marrow with increased numbers of megakaryocytes in 64% of cases. In a study conducted by Helmann et al. [107], 60% of the participants had increased bone marrow cellularity and 40% had normocellular marrow.

In our study, two patients [20%] with megakaryocytic clustering had increased bone marrow fibrosis up to grade 1 as determined by reticulin staining on a bone marrow biopsy. Similarly, in a study conducted by Gisslinger et al. [105], fibrosis was found in only 25.1 % of the cases [65 out of 259]. In another study, Hehlmann et al. [107] found that 31% of cases

had a mild increase in bone marrow fibrosis up to grade 1. These findings contradicted those of Thiele et al. [108], who found a significant increase in megakaryocyte number in all 208 cases, with the majority having grade 1 to grade 3 fibrosis on reticulin stain on bone marrow biopsy [84,13 %].

Polycythemia vera

Age and gender distribution

The patients in our study ranged in age from 22 to 76 years old. The majority of patients [6, 46.20 %] were between the ages of 61 and 70. The average age was 61 years. With a M:F ratio of 1.6:1, there was a slight male predominance. Similarly, Hassel balch et al. [111] in his study found that patients ranged in age from 19 to 87 years old, with majority in the 60 to 70 year old age group, with a mean age of 67 years and a M:F ratio of 1.2:1. Similarly, in a study conducted by Sazawal et al. [112] found that patients ranged in age from 35 to 78 years old, with the majority of patients being in their 30s and 40s, a mean age of 45 years, and a M:F ratio of 4:1. The age ranged from 18 to 83 years in a study led by Tefferi et al. [113], with a mean age of 55 years and a M:F ratio of 1.3:1. Shaikh et al. [114] conducted another study with patients aged 30-74, a mean age of 57.4 years, and a male to female ratio of 1.1:1. In their study, Usman et al. [115] found that the age varied from 20 to 65 years, the mean age was 51.3 years, and the male to female ratio was 4:1.

The current findings are in contrast to those of Cerio et al. [116], who found a small female predominance with a M:F ratio of 1:1.2, a mean age of 62 years, and an age range of 35 to 85 years. [Table 37]

Table 37: Distribution of age and gender

Authors	Mean age [years]	Range of age [years]	M:F
Hasselbach et.al[111]	67	19-87	1.2:1
Sazawal et.al[112]	45	36-78	4:1
Tefferi et.al[113]	55	18-83	1.3:1
Shaikh et.al[114]	57.4	30-74	1.1:1
Usman et.al[115]	51.3	20-65	4:1
Cerio et.al[116]	62	35.85	1:1.2
Present study	61	22-76	1.6:1

Clinical presentations and organomegaly

The most common complaints in our study were generalized weakness in 6 [46.15 %] cases and weight loss in 6 [46.15 %] cases, followed by thrombotic events resulting in symptoms such as headaches in 5 [38.46 %], vertigo in 4 [30.77 %], dizziness in 3 [23.08 %], malaise and fatigue in 4 [30.77 %] cases, paresthesia and abdominal pain in 2 [15.38 %] cases each. The results are similar to those of a study by Tefferi et al. [113], which found that the most prevalent presenting complaints were weakness [54%] and weight loss [50%] followed by thrombotic events [headaches, vertigo, dizziness, etc.] in 18% of cases. Another study by Shaikh et al. [114] found that among 58 patients, weakness and fatigue were the most common presenting complaints [50 %], followed by weight loss in 10.7% of cases and thrombotic events in 7.1 % of cases. Also in a study done by Usman et al. [115] found that 46.1 % of cases exhibited generalized weakness, with 38 % having headaches, hypertension, and other thrombotic complications.

The majority of the patients in our study showed splenomegaly [11, 84.62 %], with the majority of them having mild splenomegaly [7, 53.85 %]. Splenomegaly was found in 68.8% of patients in a study by Alessandro et

al. [117] In another study done by Macmullin et al. [118] found that splenomegaly was present in 80 % of the cases.

Hematological parameters

All of the patients in our study had hemoglobin levels of more than 16 g/dl, a high hematocrit [>49%], and a high RBC count [range from 6.87 x106/ul to 9.86 x106/ul]. Tefferi et al. [113] found that all patients had hemoglobin>15.8g/dl in their study. Thiele et al. [119] in his study found that all 362 patients had hemoglobin levels more than 16.5 g/dl. All 58 patients in another study by Shaikh et al. [114] had hemoglobin> 16.5g/dl, hematocrit >50.4 %, and an increased RBC count ranging from 6.1 to 8.8 x106/uL. Usman et al. [115] had all patients with hemoglobin of >16.2 g/dl and elevated red blood cell counts [ranging from 6.0 to 8.6x106/uL] in another study. In a study by Alessandro et al. [117], the hematocrit was greater than 53.4 % in all cases.

In our study, ten of the thirteen patients [76.92%] exhibited mild leukocytosis, with TLC ranging from 11.2 to 24.5 x103/ul. Mild leucocytosis was reported in 86.2 % of patients with TLC of >15.4x103/uL in a study by Shaikh et al [114]. Usman et al. [115] found mild leukocytosis in 76.9% of patients in his study. Our findings were in contrast to those of Alessandro et al. [117], who found that the majority of the participants [84 %] had normal TLC. Our findings were in contrast to those of Tefferi et al. [113], who found that 97 % of subjects had normal TLC and just 3% had mild leukocytosis.

In our study, thrombocytosis was found in 10 of 13 patients [76.92%], with platelet counts ranging from 477 to 1394 x103/uL. In a study done byTefferi et al. [113] found 44/63 patients [65.15 %] having thrombocytosis, with platelet counts ranging from 493 to 1345x103/uL. Also in another study conducted by Alessandro et al. [117] found that 65 % of cases had platelet counts ranging from 567 to 1248x103/uL. Another study by Shaikh et al. [114] found that all 58 patients [100%] had thrombocytosis.

Bone marrow aspiration and biopsy findings

In our study on examination of the bone marrow, all of the patients exhibited hypercellular bone marrow [100%]. The mean M:E ratio was 1:1. Megakaryocytic hyperplasia and trilineage hyperplasia were present in all of the patients. Seven cases [53.84 %] had atypical megakaryocytes, while four cases had megakaryocyte clustering [30.77 %]. In our study, fibrosis was found in only a few patients with polycythemia vera only two individuals with megakaryocytic clustering had grade 1 fibrosis, as observed on reticulin staining on bone marrow biopsy in both cases. In a study done by Thiele et al. [119] observed hypercellular marrow and trilineage hyperplasia [panmyelosis] in all 360 cases. In 60% of the cases, atypical megakaryocytes were detected, while clustered megakaryocytes were found in 45% of cases. Grade 1 fibrosis was discovered in 17.12% of the patients. Similarly in another study by Thiele et al. [120] reported all 86 patients [100%] had panmyelosis. Megakaryocytic hyperplasia was found in 98% of patients, with pleomorphic and atypical megakaryocytes and clustering in 51% of cases. Only 6 out of 86 cases [6.97%] exhibited reticulin fibrosis that was grade 1. [Table 38]

Table 38: Bone marrow and megakaryocytic findings

Authors	% of patients with panmyelosis	% of atypical megakaryocytes	% of megakaryocytic clustering	Grade of fibrosis on bone marrow biopsy [%]
Thiele et.al[119]	100%	60%	38%	Grade 1 [17.12%]
Thiele et.al[120]	100%	98%	51%	Grade 1 [6.97%]
Present study	100%	53.84%	30.77%	Grade 1 [15.38%]

Primary myelofibrosis

Age and gender distribution

Primary myelofibrosis was shown to be more frequent in the older age group in the current study. The age ranged from 46 to 73 years old, with the bulk of patients [60 %] being between the ages of 61 and 70. With a M:F ratio of 1.5:1, there was a little male predominance. Patil et al. [121] in his found that 29/50 patients were male and 21 were female, with a M:F ratio of 1.3:1. The most common age group was 60-70 years. Cervantes et al. [122] found that the most common age group was 61-70 years old, with a M:F ratio of 1.5:1.

Clinical presentations and organomegaly

The most prevalent presenting complaints in our study were generalized weakness [70%], fever [60%], abdominal pain [40%], and weight loss [40%], all of whom showed organomegaly on physical examination. Malaise and fatigue were found in two [20%] of the patients, while night sweats were seen in two [20%] of the patients.

The findings differed from those of Patil et al., they reported that the most prevalent symptom of the presentation was pain/discomfort in the abdomen [owing to splenomegaly] in 34 out of 50 cases [68 %], followed by fatigue or weakness in 25 out of 50 cases [50 %]. In another study by Tefferi et al. [123] observed that weakness was seen in only 29% of cases, fever in 30%, and stomach pain caused by splenomegaly in 80% of cases. Our findings are also in line with WHO Hematopoietic and Lymphoid Tissues, 2017 revised edition [124], which mentions constitutional symptoms [weakness, fatigue, weight loss, etc.] in 50% of cases, splenomegaly in 90% of cases, and hepatomegaly in 50% of cases.

Organomegaly was found in all of the patients with primary myelofibrosis in our study. Splenomegaly was seen in 8 people [80%], with the majority having moderate to massive splenomegaly [7, 70%], and hepatomegaly in

8 people [80%]. Splenomegaly was detected in 89 % of cases and hepatomegaly was seen in 50 % of cases in a research by Cervantes et al. [122]. J.Thiele et.al [125] reported splenomegaly in 80% of cases and hepatomegaly in 60% of cases. In another study by Patil et al. [121] reported splenomegaly was seen in 100% of the patients [50/50 patients]. Tefferi et al. [123] also observed that 72.8 % of people had moderate to large splenomegaly.

Hematological parameters

The majority of patients in our study had anemia, with 7 [70%] had moderate anemia ranging from 8.1 to 10.7 g/dl and 2 [20%] had severe anemia with Hb 7.6 and 5.6 g/dl, respectively. The majority of the patients showed microcytic hypochromic anemia, with nRBCs seen on peripheral blood film.

In a study conducted by J.Thiele et al. [125], significant anemia and a leucoerythroblastic blood picture were detected in all 60 patients [100%]. Barosi et al. [126] concluded that the majority of patients [80.7%] had mild anemia with a leucoerythroblastic appearance. In another study by Tefferi et al. [123] observed that mild anemia was a common finding in 38 % of patients.

In our study six [60%] patients had normal range TLC ranging from 4.6 to 10.4 x103/uL, while three [30%] patients showed variable leukocytosis TLC ranging from 23.8 to 135x103/ul. In a study by Barosi et al. [126] they reported that the majority of patients [64.3 %] had normal range TLC and just 25% had leukocytosis. The results of our study are in contrast to those of Patil et al. [121], they observed mild leukocytosis in all 50 cases [100%] with TLC of >15.3x103/ul. In another study done by Tefferi et al. [123] reported that the majority of people had normal TLC levels, with just 13% having leukocytosis [TLC >11x103/uL].

In our study, the majority of the patients [70 %] had normal platelet counts ranging from 172 to 404x103/uL. Thrombocytopenia was found in the

remaining three [30%] patients, with platelet counts ranging from 80 to 111x103/ul. In a study done by Cervantes et al. [122] concluded that 53 % had a normal platelet count, 30.5 % had thrombocytosis, and 16.5 % had thrombocytopenia. Also in another study done by Tefferi et al. [123] reported that the majority of people had normal platelet counts, with only 18% having thrombocytopenia.

Bone marrow aspiration and biopsy findings

In our study 50% patients had hypocellular marrow with decreased erythropoiesis [60%] and myelopoiesis was in [70%]. Megakaryocytic hyperplasia was seen in the majority of cases [70%], with clustering seen in 5 cases [50%]. Grade 1 to grade 3 fibrosis was detected in all cases, with grade 3 fibrosis in 4 cases [40%], grade 2 in 2 cases [20%], and grade 1 in 4 cases [40%]. Findings were concordant with study conducted by Pereira et al. [127] they reported that all 51 cases [100%] showed megakaryocytic hyperplasia, with Grade 3 fibrosis in 54.9 %, grade 1 to grade 2 in 45 %. Similarly Poala et al. [128] also observed that 36.7 % of the patients had fibrosis grade 1, 40.0 % had grade 2, and 23.3 % had grade 3.

SUMMARY AND CONCLUSION

The present study was conducted in Department of Pathology at Dayanand medical college and hospital, Ludhiana, for a period of five years on hematological and genetic profile of myeloproliferative neoplasms.During this period a total of 4438 bone marrow aspirations done, 138 were confirmed to have genetic mutational analysis of myeloproliferative neoplasm [CML, ET, PV, PMF] and were analysed and relevant data was compiled. The following features were highlighted in the study.

- The age of the patients ranged from 7 to 81 years with mean age of 58 years. The majority of patients were in age group of 41 to 50 years [35 cases, 25.4%]. Overall slight male predominance was noted with M:F was 1.3:1.
- Molecular analysis revealed that 105 cases had a BCR ABL fusion gene [83 ,79.04 % had 210kD while 22 ,20.95 % had both 210kD and 190kD]. 33/138 were BCR ABL negative but JAK2v617f positive and were classified based on the clinical presentation, hemogram, bone marrow biopsy and aspiration findings intoET [10/33 cases], PV [13/33 cases],PMF [10/33 cases].
- The common presenting complaints were generalized weakness [92, 66.7%], followed by malaise and fatigue [57, 41.30%], weight loss [51, 37.00%], fever [37, 26.80%]. Majority of patients presented with splenomegaly [mild in 23.2% cases, moderate in 51.2% cases, massive in 25.6% cases].
- Amongst all the 105 CML cases, 102 were in chronic phase, 2 were in accelerated phase and 1 was in blast crisis. Majority of patients [54, 50.49%] presented with moderate degree of anaemia [Hb 8.1-10.9 gm/dl] andvarying degree of leucocytosis.
- Age adjusted hypercellularity with myeloid hyperplasia was seen in all of the cases of CML with mean M:E ratio of 8:1. Megakaryocytes

were increased in 95/105 cases, [16 had hyperlobated and 58 had dwarf forms]. 21 cases [20%] had grade 1 to grade 3 fibrosis.

- Essential thrombocythemia [10 cases, 9.42%] had M:F ratio of 1.5:1. These patients had peripheral thrombocytosis [>450x10^3/uL] with normal range haemoglobin andhematocrit. Mild leukocytosis was seen in 6/10 patients, 60.0%. all cases had increased number of megakaryocytes with presence of atypical and hyperlobated nuclei in 80% and clustering of megakaryocytes 20% cases. Fibrosis was uncommon with only 2 patients had grade 1 fibrosis on reticulin stain.
- Polycythemia vera [13 cases, 9.42%] had M:F of 1.6:1. Majority patients [10 cases, 76.92%] had thrombocytosis [477 to 1394 x10^3/uL]. 100% patients had Hb levels of > 16 g/dl with raised RBC count [6.87x10^6/ul - 9.86 x10^6/uL] and hematocrit [>49%]. On bone marrow examination all patients had hypercellular marrow with trilineage hyperplasia and megakaryocytic hyperplasia with atypical megakaryocytes [7, 5.84] and clustering of megakaryocytes [4, 30.77%]. Fibrosis was an uncommon with only 2 patients had grade 1 fibrosis on reticulin stain.
- Primary myelofibrosis [10 cases, 7.25%] had M:F of 1.5:1. Splenomegaly was common in 8 patients [80%]. Majority patients had moderate anemia in 7 patients [70%]. On bone marrow examination megakaryocytic hyperplasia was noted in 7 cases [70%] and clustering in 5 cases [50%] cases. Grade 1 to grade 3 fibrosis was seen in all cases with grade 3 fibrosis in 4 [40%], grade 2 in 2 [20%] and grade 1 in 4 [40%] on reticulin stain.
- So, in conclusion cytogenetics is essential in making diagnosis of myeloproliferative neoplasm. But, there is also great role of hemogram and bone marrow aspiration and biopsy in further classification of these neoplasms.

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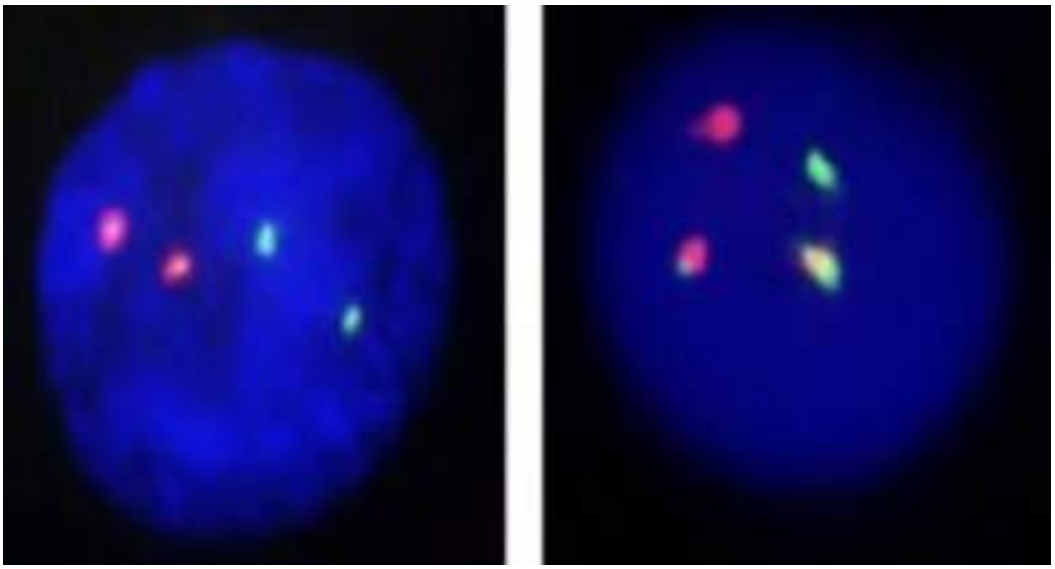
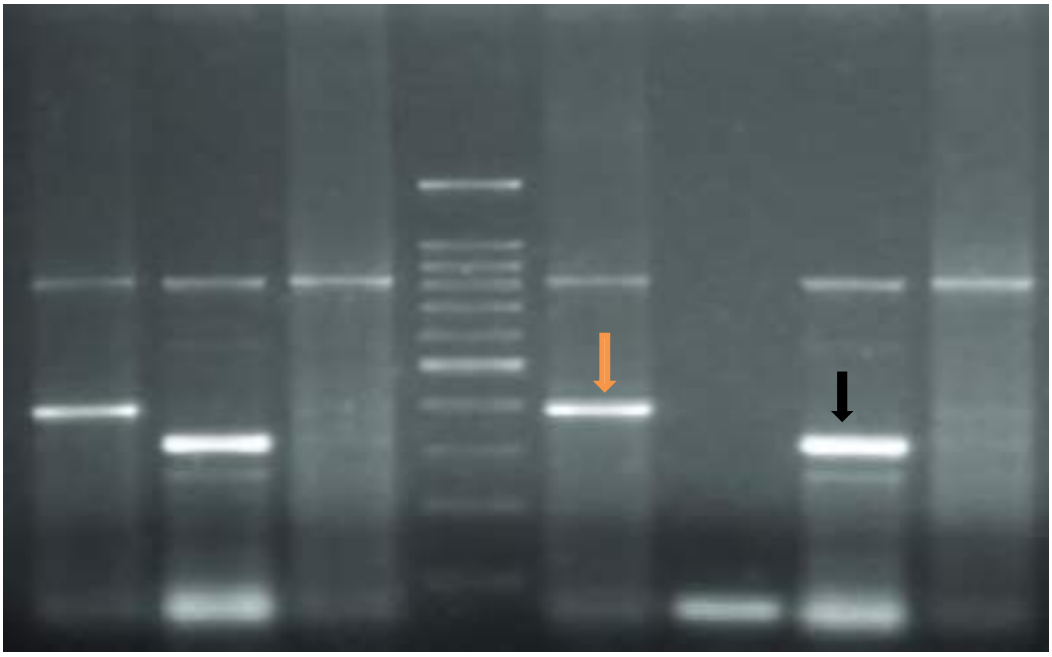
PICTURES

Picture 1: Bio-Rad Real Time PCR system

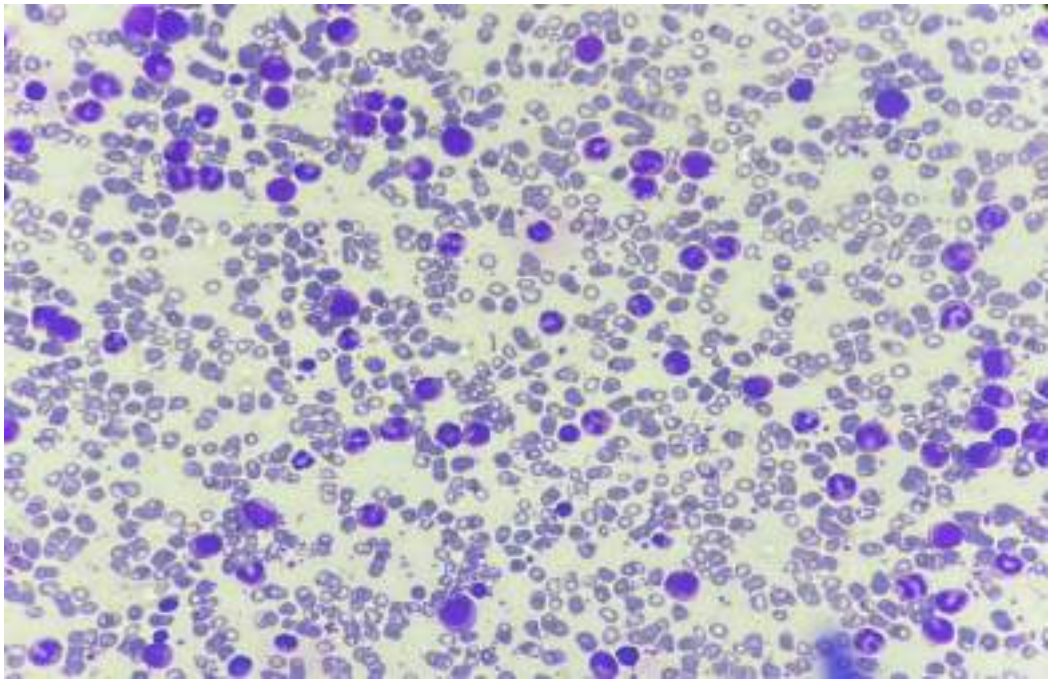
Picture 2: Olympus BX61 FISH instrument



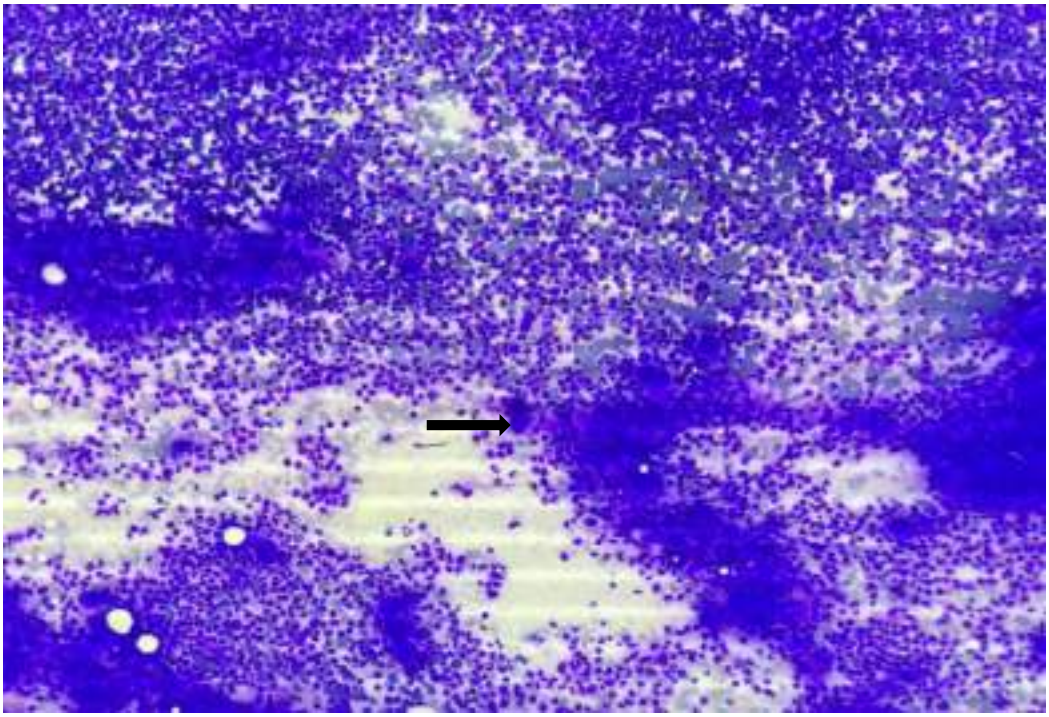
Picture 3: FISH- 2 red dots are chromosome 9 and green is chromosome 22.The yellow dot in next image is fusion of 9:22 (BCR ABL).



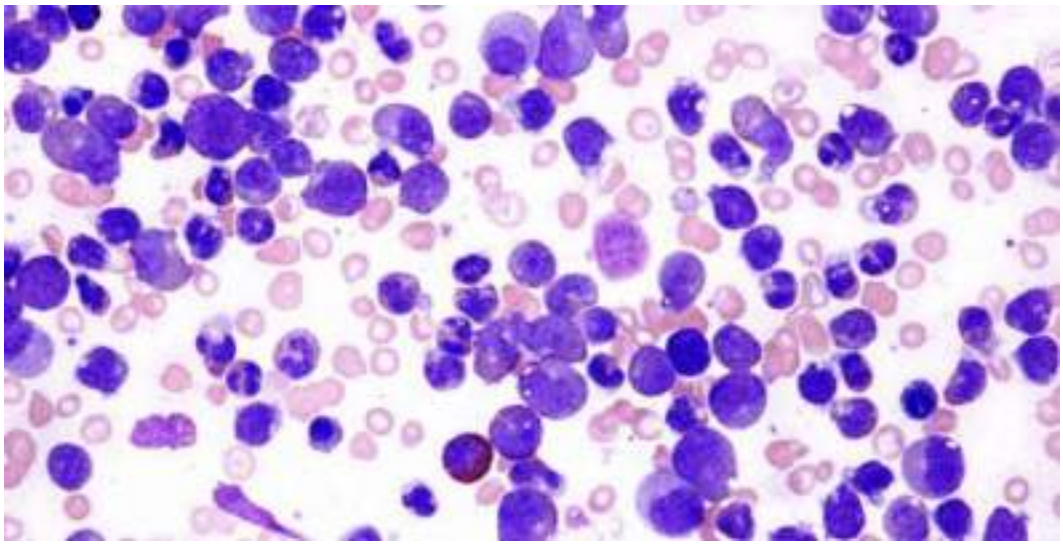
Picture 4: BCR-ABL transcripts are seen post RT PCR via gel electrophoresis.The bright white signal depicts presence of BCR ABL fusion gene. [Black arrow- patient's sample, yellow arrow- positive control]



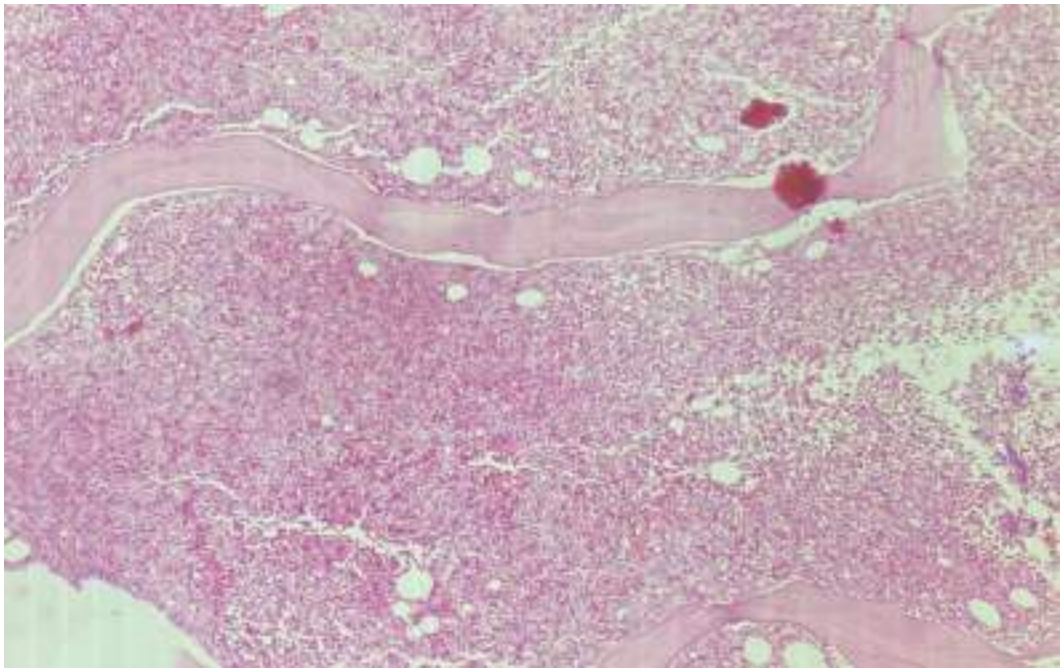
Picture 5: CML in chronic phase. Peripheral blood smear shows leukocytosis with granulocytic cells in various stages of maturation. [MGG, 400x]



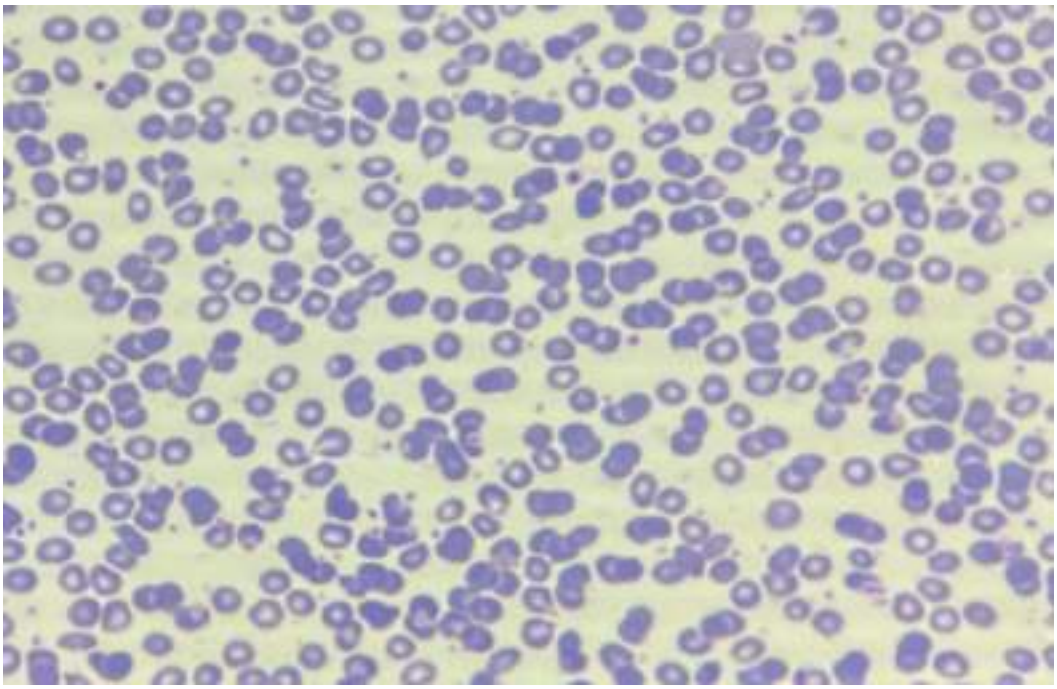
Picture 6: CML in chronic phase. Bone marrow aspirate shows hypercellular marrow with marked myeloid hyperplasia along with prominent increase in number of dwarf megakaryocytes [black arrow].[MMG, 100x]



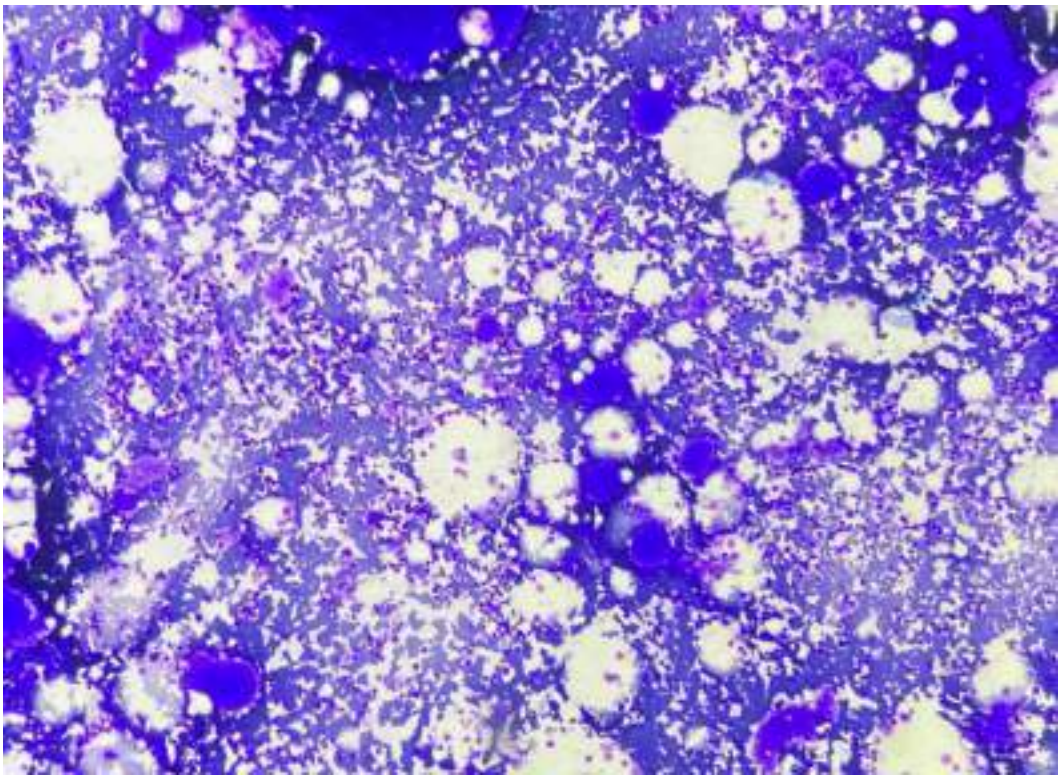
Picture 7: CML in chronic phase. Bone marrow aspirate shows hypercellular marrow with marked myeloid hyperplasia with orderly cell maturation[MGG, 1000x]



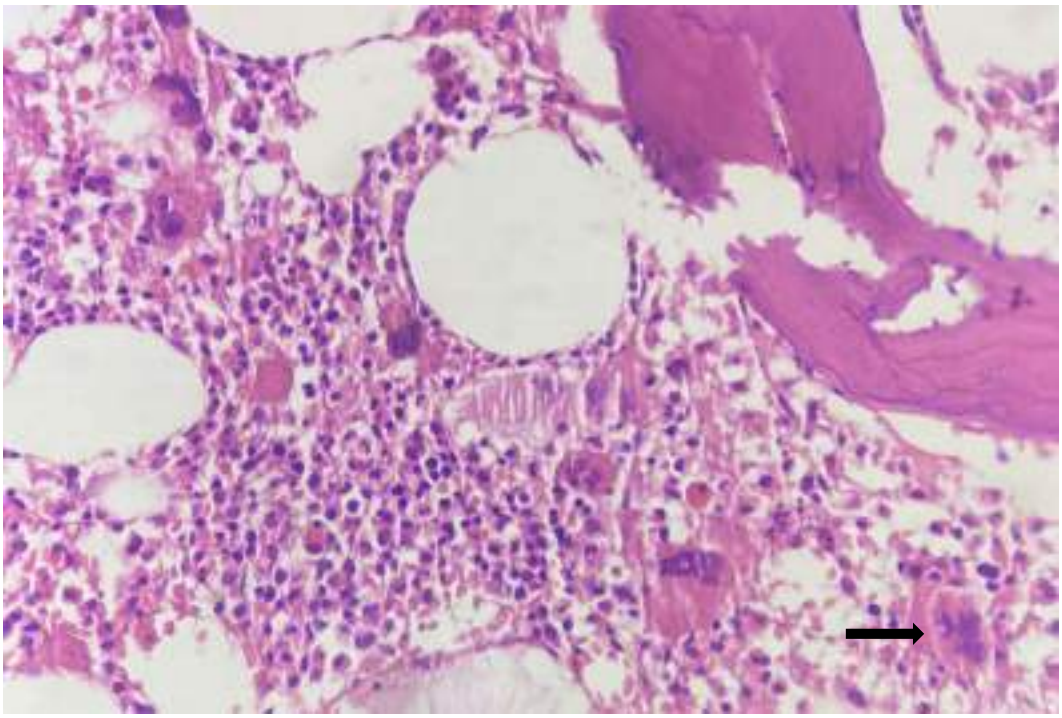
Picture 8: CML, Bone marrow biopsy shows hypercellular marrow with marked myeloid hyperplasia. [H&E, 100x]



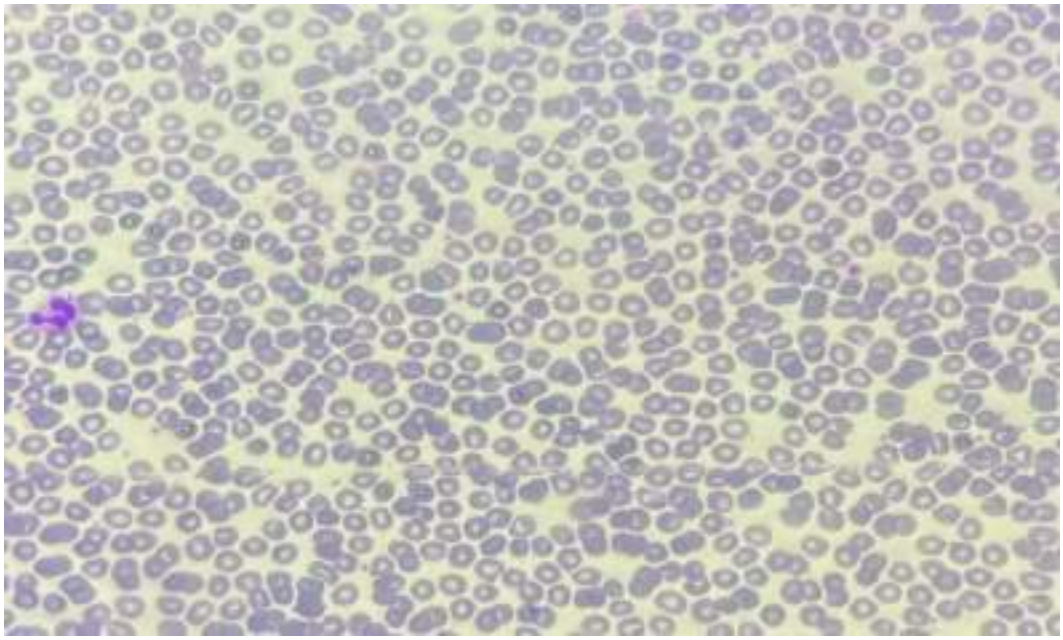
Picture 9: Essential thrombocythemia. PBF shows thrombocytosis. [MGG, 400x]



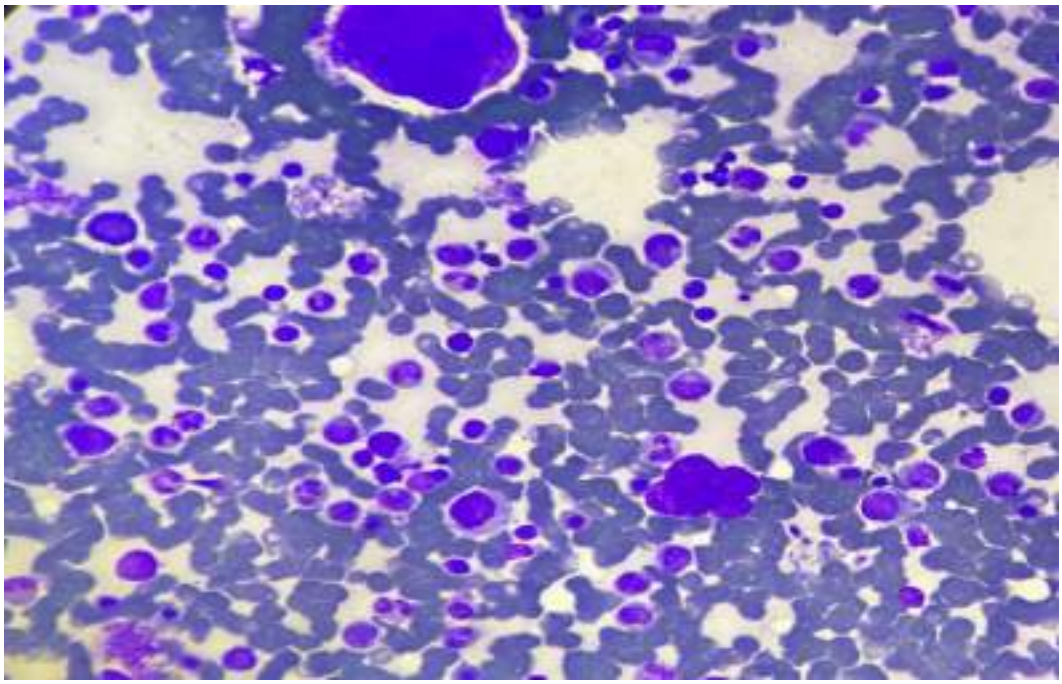
Picture 10: Bone marrow aspirate shows megakaryocytic hyperplasia with hypolobated and hyperlobated forms. [MGG, 100x]



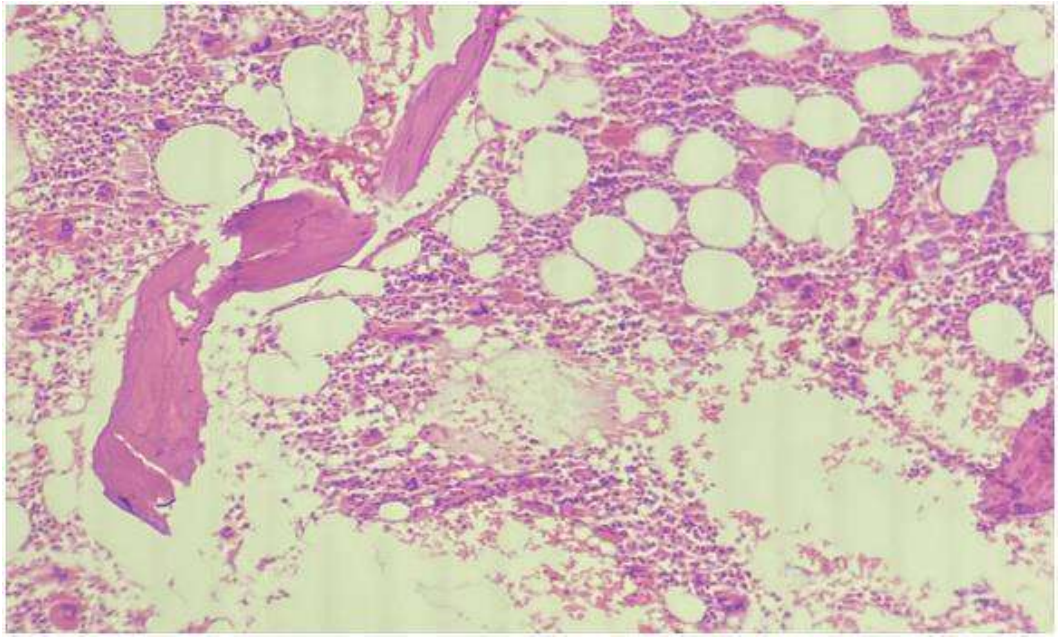
Picture 11: ET, bone marrow biopsy shows megakaryocytic hyperplasia with clustering. Megakaryocytes are large with hyperlobated nuclei [Black arrow- staghorn nuclei].[H&E, 400x]



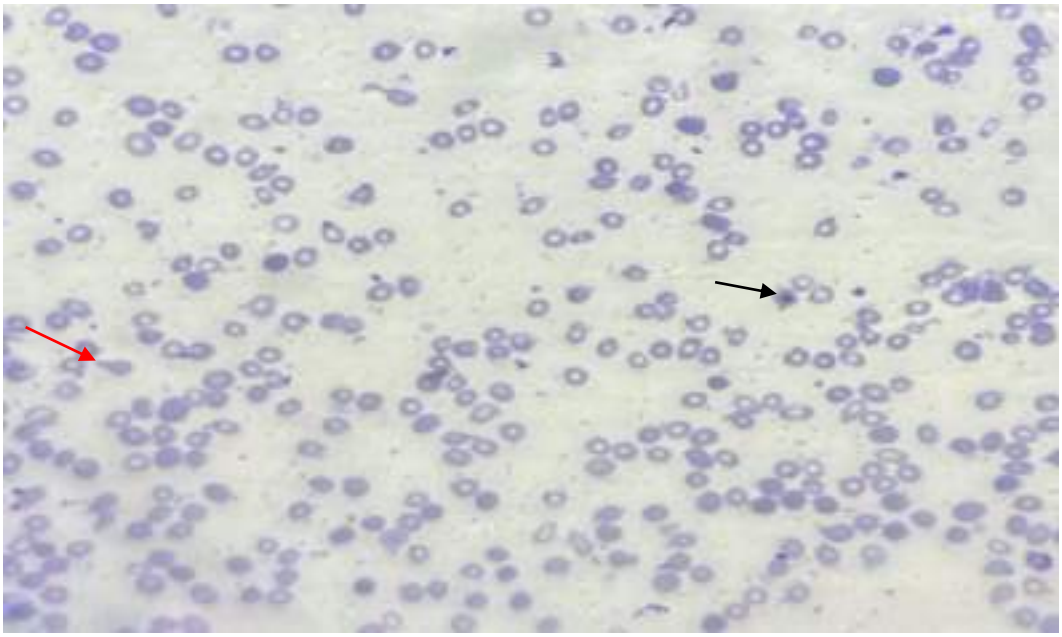
Picture 12: PV. PBF shows closed packed red blood cells. [MGG, 100x]



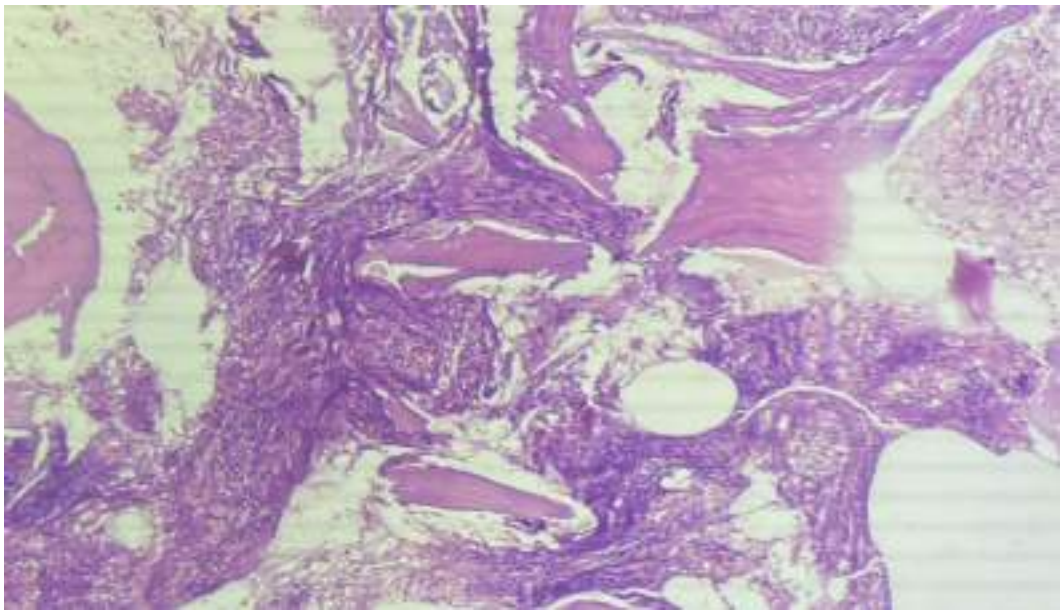
Picture13: PV. Bone marrow aspiration shows pleomorphic megakaryocytes. [MGG, 400x]



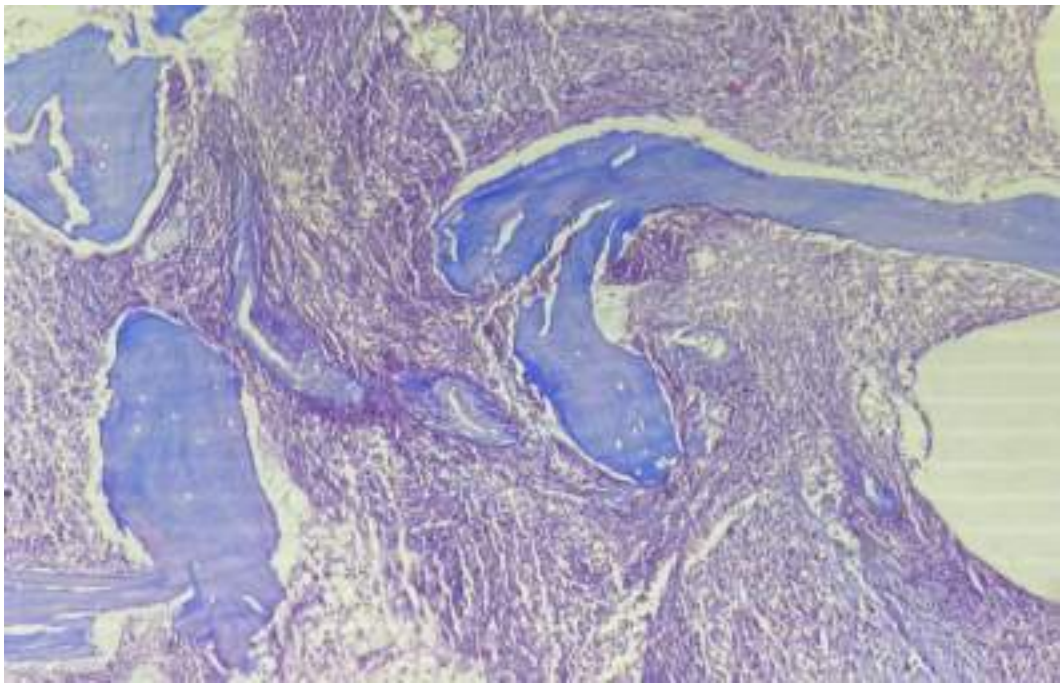
Picture 14: PV. Bone marrow biopsy shows hypercellular marrow with panmyelosis and pleomorphic megakaryocytes. [H&E, 100x]



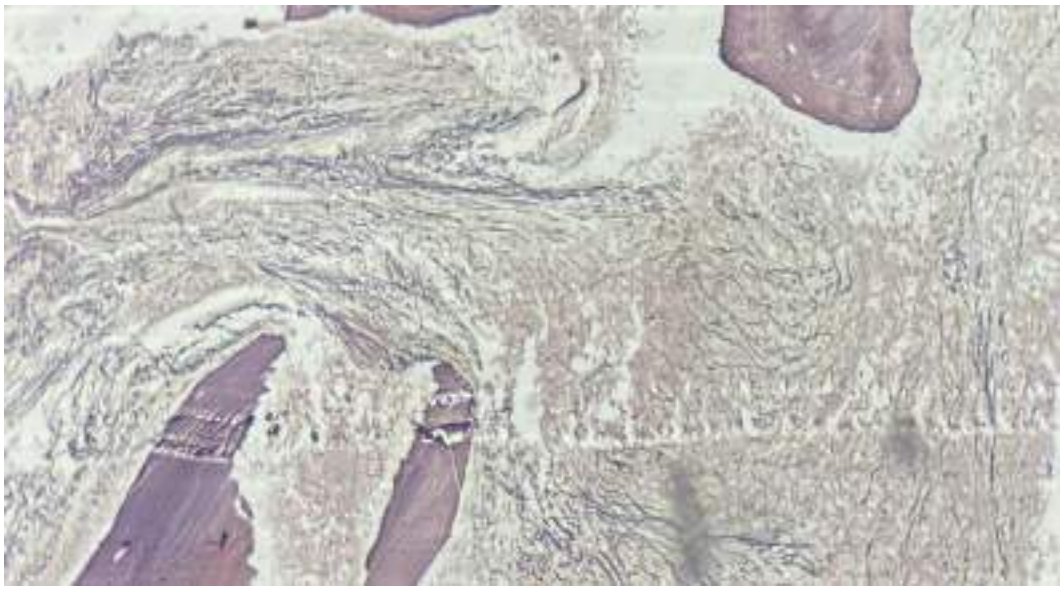
Picture 15: PMF. Peripheral blood film shows tear drop cells [red arrow] with microcytic RBCs and a nucleated RBC [black arrow].[MGG, 100x]



Picture 16: Bone marrow biopsy shows diffuse and dense increase in reticulin fibre. [H&E, 100x]



Picture 17: Bone marrow biopsy shows diffuse and dense increase in reticulin fibres with extensive intersections and coarse bundles of thick collagen [MT, 100x].



Picture 18: Bone marrow biopsy shows diffuse and dense increase in reticulin fibres with extensive intersections. [Reticulin, 100x].

						CLINICAL HISTORY- chief complaints														GENERAL PHY	
S.NO	MRD NO.	HEMAT.NO	NAME	AGE	SEX	generalised weakness	malaise and fatigue	weight loss	fever	night sweats	abdominal pain	paresthesia	vertigo	headaches	dizziness	bleeding tendencies	bluish discolouration of digits	DURATION	PERSONAL HISTORY	Appearance	SPLEENOMEGALY
1	514845	18-529	SK	51	m	present	present	-	-	-	-	-	-	-	-	-	-	2months	occasional smoker reformed alcoholic	pallor	moderate
2	437885	18-132	NV	31	m	-	present	-	present	-	-	-	-	-	-	present	-	1month	-	pallor	massive
3	830523	19-12778	HS	46	m	present	-	-	-	-	-	-	-	-	-	-	-	10days	occasional drinker	pallor	mild
4	848379	19-13700	AP	68	m	present	-	-	-	-	-	-	-	-	-	present	-	20days	H/O pulmonary TB	pallor	moderate
5	514845	18-529	SS	48	m	present	-	present	-	-	-	-	-	-	-	-	-	1month	-	pallor	no
6	410487	18-25	N	22	f	present	present	present	-	-	-	-	-	-	-	-	-	15days	-	-	massive
7	440037	18-139	J	60	m	-	-	-	-	-	present	-	-	-	-	-	-	1.5years	chronic tobacco -reformed	pallor	massive
8	464049	18-270	BS	76	m	-	present	-	-	-	-	-	-	-	-	-	-	1month	-	pallor	moderate
9	459637	18-271	SS	70	m	present	-	present	-	-	present	-	-	-	-	-	-	-	DM HTN alcoholic	pallor	mild
10	416684	18-426	MS	50	m	present	-	present	-	-	-	-	-	-	-	-	-	20days	desi medication intake	pallor	moderate
11	478613	18-488	RK	45	f	-	-	present	present	-	-	-	-	-	-	-	-	15days	DM HTN TB constrictive pericarditis	pallor	moderate
12	502886	18-492	BK	64	f	present	-	present	-	-	-	-	-	-	-	-	-	10 days	-	pallor	no
13	518938	20-59	KS	34	m	present	-	-	-	-	-	-	-	-	-	present	-	5 days	k/c/o CML	pallor	moderate
14	58732	18-655	DK	60	f	present	-	present	-	-	-	-	-	-	-	-	-	-	-	pallor	massive
15	540424	18-757	KR	46	f	-	-	present	-	present	-	-	-	-	-	-	-	1 month	desi medication intake	pallor	mild
16	118749	18-773	KN	67	f	present	-	present	present	-	-	-	-	-	-	-	-	1.5month	-	pallor	moderate
17	604233	18-1079	HC	70	m	present	-	-	present	-	-	-	-	-	-	-	-	3 months	smoker	pallor	moderate
18	610963	18-1031	GS	43	m	present	present	-	-	-	-	-	-	-	-	-	-	2 months	smoker & alcohol	pallor	moderate
19	467195	18-1016	BS	73	m	present	-	-	-	-	present	-	-	-	-	-	-	-	DM HTN, urethral stricture	pallor	massive
20	540259	19-557	RK	68	f	present	-	present	-	-	-	-	-	-	-	-	-	1month	-	-	massive
21	546682	18-704	DM	58	m	present	present	-	present	-	-	-	-	-	-	-	-	2months	HTN	-	moderate
22	275365	18-729	GS	55	m	-	present	-	-	-	-	-	-	-	-	-	-	1.5months	ocaasional drinker	pallor	moderate
23	396093	18-770	SS	81	m	-	-	-	-	-	-	-	-	-	-	-	present	1month	-	pallor	mild
24	579606	18-865	RD	29	f	-	present	-	-	-	-	-	-	-	-	-	-	2months	-	pallor	mild
25	635586	18-1163	RK	52	f	present	-	-	present	-	-	-	-	-	-	-	-	1month	hypothyroidism	pallor	massive
27	659530	19-81	KS	69	m	present	-	present	-	-	-	-	-	-	-	-	-	15days	DM	pallor	moderate
28	721990	19-391	RS	18	m	-	present	-	-	-	-	-	-	-	-	-	-	1month	-	pallor	no
29	763211	19-634	MK	30	f	-	present	present	-	-	-	-	-	-	-	-	-	2weeks	-	pallor	moderate
30	78114	19-749	SK	38	f	present	-	-	-	-	-	-	-	-	-	-	-	1months	-	pallor	massive
31	922657	20-312	RG	26	m	-	-	present	present	-	-	-	-	-	-	-	-	2months	-	pallor	moderate
32	921895	20-304	RK	49	f	-	present	-	-	-	-	-	-	-	-	-	-	4weeks	-	pallor	mild
33	928854	20-379	B	57	m	present	present	-	-	present	-	-	-	-	-	-	-	3weeks	-	-	moderate
34	723056	19-417	HS	36	m	present	-	-	present	-	-	-	-	-	-	-	-	6month	-	-	mild
35	723848	19-410	RS	61	m	-	-	-	-	-	-	-	present	present	-	-	-	5month	DM HTN	plethora	mild
36	741476	19-499	A	42	f	present	-	present	present	-	present	-	-	-	-	-	-	4month	-	-	mild
37	605344	19-204	PK	42	f	-	-	-	-	-	present	-	present	present	present	-	present	2months	DM	-	mild
38	483768	19-845	PK	43	m	present	-	present	-	-	-	-	-	-	-	-	-	3weeks	DM HTN	-	mild
39	838772	19-1197	MS	69	m	-	-	-	-	-	-	-	-	present	-	-	-	6 months	desi medication intake	-	no
39	766389	19-661	SS	56	m	-	-	-	-	-	-	-	-	present	-	-	-	2months	HTN	pallor	mild
40	900201	20-158	ARG	49	f	-	present	-	-	-	-	-	-	-	-	-	-	1.5month	-	-	moderate
40	864482	19-1210	MS	48	m	present	-	present	-	-	-	-	-	-	-	-	-	2months	-	pallor	moderate
41	699164	19-265	JK	68	f	present	present	present	-	present	-	-	-	-	-	-	-	5months	-	pallor	moderate
42	920101	20-262	PK	34	f	present	present	-	-	-	-	-	-	-	-	-	-	1.5month	-	-	moderate
43	690257	19-217	AS	38	m	-	present	-	present	-	-	-	-	-	-	-	-	1month	-	-	mild
44	462539	18-254	AS	28	m	present	present	present	-	-	-	-	-	-	-	-	-	10days	-	pallor	mild
45	632143	19-94	MK	52	f	present	-	present	-	-	present	-	-	-	-	-	-	1.5months	-	-	no
46	924802	20-583	HS	42	m		-	-	-	-	-	present	present	present	present	-	-	2months	-	-	moderate
47	914905	20-252	AS	70	f	-	-	-	present	-	-	-	present	present	-	-	-	1month	-	-	no
48	780561	19-761	P	7	f	present	-	present	-	-	-	-	-	-	-	-	-	-	-	-	no
49	538156	18-658	L	27	m	-	-	present	present	-	-	-	-	-	-	-	-	-	-	pallor	mild
50	116967	16-676	MS	32	f	present	-	-	-	-	-	-	-	-	-	present	-	1month	pemphigus vulgaris	pallor	no
51	625969	18-1115	JR	48	m	-	present	-	-	-	-	-	-	-	-	-	-	6months	H/O TB	pallor	moderate
52	687624	19-207	MS	70	m	present	present	-	-	-	-	-	-	-	-	-	-	6months	-	pallor	moderate
53	712297	19-1262	BK	50	f	present	-	present	-	-	-	-	-	-	-	-	-	2months	-	-	mild
54	558937	18-783	AS	50	m	present	-	-	-	-	-	-	-	-	-	-	-	2weeks	-	pallor	moderate
55	557964	18-769	SS	61	m	present	-	-	present	-	present	-	-	-	-	-	-	-	-	pallor	no
56	456615	18-837	BS	58	m	present	present	-	-	-	-	-	-	-	-	-	-	2.5months	-	pallor	moderate
57	578510	18-862	TG	29	m	present	-	present	-	-	-	-	-	-	-	-	-	3weeks	-	-	mild
58	453845	18-900	RMS	70	m	present	-	present	-	-	-	-	-	-	-	-	-	1 month	rheumatoid arthritis	-	no
59	608482	18-1008	A	30	f	present	-	-	-	-	-	-	-	-	-	-	-	3months	-	pallor	massive
60	642490	19-61	CK	30	f	present	-	present	-	-	-	-	-	-	-	-	-	2months	-	pallor	mild
61	452833	18-212	PK	39	f	present	-	-	-	-	-	-	-	-	-	-	-	3months	-	pallor	massive
62	672654	19-1695	KD	72	f	-	present	-	present	-	-	-	-	-	-	-	-	2months	DM	-	moderate
63	621139	18-1071	AK	43	m	present	-	-	present	-	-	-	-	-	-	-	-	-	-	-	mild
64	556268	18-746	PM	31	m	present	-	-	-	-	-	-	-	-	-	-	-	3weeks	-	pallor	moderate
65	238274	19-230	DS	61	m	present	-	-	-	-	-	-	-	-	-	-	-	2 motnh	k/c/o CML	-	mild
66	697511	19-257	G	31	m	-	-	-	present	-	present	-	-	-	-	-	-	10days	-	pallor	moderate
67	715831	19-362	MS	65	m	present	-	-	present	-	present	-	-	-	-	-	-	15days	-	-	mild
68	805503	19-893	BK	31	m	-	present	present	-	-	-	-	-	-	-	-	-	1month	occasional drinker	-	moderate
69	816910	19-948	GK	52	f	-	present	-	-	-	-	-	-	-	-	-	-	-	-	-	moderate
70	905162	20-181	HK	29	f	present	present	present	-	-	-	-	-	-	-	-	-	-	-	pallor	no
71	932581	20-412	GS	24	m	present	present	-	-	-	-	-	-	-	-	-	-	-	-	pallor	moderate
72	936634	20-451	BS	39	m	present	-	-	-	-	-	-	-	-	-	-	-	-	chronic alcohol intake	-	moderate

		SICAL EXAMINATION		HEMATOLOGICAL PARAMETERS																		
S.NO	MRD NO.	HEPATOMEGALY	lymphadenopathy	TLC	neutrophil	lymphocyte	monocyte	eosinophil	basophil	promyelocyte	myelocyte	metamyelocyte	blast	PLATELETS	HG g/dl	nrbcs	PBF	hematocrit	rbc count	MCV	CELLULARITY
1	514845	mild	no	177	34	3	0	0	0	0	35	26	2	391	7.7	present	normocytic with tear drop cells	36.7	3.2	83	hypercellular
2	437885	no	no	258.7	40	3	3	3	1	0	22	22	5	593	10.6	present	normocytic	34.1	3.82	89.3	hypercellular
3	830523	mild	no	76.4	64	15	3	2	1	0	15	8	2	510	8.5	present	microcytic	29.8	2.89	74.2	hypercellular
4	848379	no	present	4	66	30	4	0	0	0	0	0	0	32	7.6	-	normocytic	26.7	2.1	78.9	no fragments
5	514845	no	no	31	42	3	0	0	3	8	20	19	5	495	8.1	present	normocytic	38.7	3.59	80.7	hypercellular
6	410487	moderate	no	11.5	85	8	6	1	0	0	0	0	0	1394	18.6	-	normocytic	49.8	6.87	89.8	hypercellular
7	440037	no	no	10.4	55	26	4	0	0	0	10	15	0	285	10.7	-	normocytic	34	4.3	79.2	dry tap
8	464049	no	no	24.5	87	7	3	3	0	0	0	0	0	350	15.5	-	normocytic	56.3	6.78	82.1	hypercellular
9	459637	mild	no	23.9	85	10	2	3	0	0	0	0	0	2750	10.1	present	normocytic	33.8	5.03	67.1	hypercellular
10	416684	mild	no	126.9	44	3	0	3	4	0	13	30	3	462	8.7	-	normocytic	32.6	3.61	82.7	hypercellular
11	478613	moderate	present	311.3	43	4	1	2	1	4	14	20	4	660	10.1	present	microcytic	24.3	3.52	76.4	hypercellular
12	502886	mild	no	4.6	75	10	6	0	0	0	2	2	0	111	10.7	present	microcytic tear drop cells	34.8	4.92	70.7	no fragments
13	518938	no	no	26.5	54	20	3	9	2	1	6	4	1	120	6.9	-	normocytic	36.4	2.16	83.4	hypercellular
14	58732	moderate	no	6	63	33	4	0	0	0	0	0	0	1802	10.8	-	microcytic tear drop cells	14	1.76	79.7	scanty fragments
15	540424	mild	no	8.1	78	12	9	1	0	0	0	0	0	292	9.9	present	microcytic tear drop cells	39	3.23	67	no fragments
16	118749	mild	no	3	74	9	10	5	0	0	1	1	0	100	8.1	present	microcytic tear drop cells	24.7	2.64	63.9	hypercellular
17	604233	mild	no	23.8	79	10	3	8	0	0	0	0	0	404	9.4	present	microcytic tear drop cells	32.6	5.71	57	hypercellular
18	610963	moderate	no	111.9	63	4	3	2	3	2	10	10	3	497	9.1	present	microcytic	40.9	2.59	71.1	hypercellular
19	467195	mild	no	10	84	10	3	0	0	0	2	1	0	251	7.6	present	microcytic tear drop cells	23	2.89	79.6	hypercellular
20	540259	massive	no	54.3	68	2	1	2	0	0	10	15	2	134	13.6	-	macrocytic	38.7	3	92	hypercellular
21	546682	moderate	no	135	87	3	0	2	0	0	4	4	0	341	12.9	-	normocytic	36.2	4.19	86.5	hypercellular
22	275365	moderate	no	264	17	3	2	2	0	0	41	34	1	150	6.5	-	normocytic	24.5	2.89	89.7	hypercellular
23	396093	mild	no	10.7	81	11	7	1	0	0	0	0	0	1201	9.8	-	normocytic	30.3	3.37	89.9	hypercellular
24	579606	mild	no	54.9	24	6	3	2	2	5	10	10	38	550	8.9	present	microcytic	31.5	2.99	76.9	hypercellular
25	635586	moderate	no	302.9	40	2	1	2	4	0	22	27	2	658	2.7	present	microcytic	43.7	2.99	74	hypercellular
27	659530	moderate	no	338	39	2	0	5	1	1	20	25	7	460	10.1	present	normocytic	40.7	3.9	90.6	hypercellular
28	721990	mild	no	35.9	77	5	8	2	0	0	5	4	0	564	9.7	-	microcytic	38.9	3.28	70.9	hypercellular
29	763211	massive	no	167.3	66	3	0	1	2	0	15	10	3	464	10.6	-	normocytic	43.2	3.98	94	hypercellular
30	78114	moderate	no	394	9	0	0	2	0	0	50	29	7	488	9	present	microcytic	35	3.65	65	hypercellular
31	922657	moderate	no	287.6	54	1	3	6	0	0	10	15	3	171	9.9	present	normocytic	36.4	4.1	94	hypercellular
32	921895	moderate	no	111	63	4	3	2	3	0	15	5	3	497	9.1	present	microcytic	42.3	4.22	69	hypercellular
33	928854	no	no	105.2	52	6	2	3	2	2	20	18	5	204	13.3	present	normocytic	36.8	4.68	86	hypercellular
34	723056	mild	no	13.2	81	6	7	6	0	0	0	0	0	1071	12.7	-	normocytic	32.3	3.24	80.1	hypercellular
35	723848	no	no	17.5	78	16	5	1	0	0	0	0	0	723	17.5	-	normocytic	55.4	7.98	79.6	hypercellular
36	741476	moderate	no	13.6	71	14	8	4	0	0	1	2	0	2708	13.6	-	normocytic	27.6	3.65	82.6	hypercellular
37	605344	no	no	11.2	80	16	3	1	0	0	0	0	0	897	15.7	-	normocytic	58.9	7.65	84.3	hypercellular
38	483768	mild	no	11.4	81	15	4	0	0	0	0	0	0	1142	16	-	normocytic	49.9	6.98	85.6	hypercellular
39	838772	mild	no	20.5	70	24	5	1	0	0	0	0	0	619	16.9	-	normocytic	57.6	6.99	83.9	hypercellular
39	766389	mild	no	37.8	69	8	1	5	1	0	10	15	1	523	9.5	present	microcytic tear drop cells	39.7	3.98	61	hypercellular
40	900201	no	no	50	68	4	6	2	0	0	10	10	0	605	12.8	present	microcytic	38.9	4.65	69.1	hypercellular
40	864482	no	no	179	46	2	15	0	2	0	15	30	5	610	10.1	present	normocytic	40.5	4.01	79.8	hypercellular
41	699164	moderate	no	334	46	5	3	7	1	0	25	10	3	517	9.7	-	microcytic	41.3	3.98	62.4	hypercellular
42	920101	mild	no	59	58	5	0	2	1	0	18	7	8	619	12.9	present	normocytic	42.7	4.21	90	hypercellular
43	690257	no	no	71.5	70	12	2	2	2	0	8	2	2	221	12.7	present	normocytic	44.6	4.23	91	hypercellular
44	462539	mild	no	37.8	69	8	1	5	1	0	10	5	1	223	9.5	present	microcytic	42.1	3.02	78	hypercellular
45	632143	moderate	no	21.9	84	10	2	4	0	0	0	0	0	796	14.9	-	normocytic	54.7	7.95	84.3	hypercellular
46	924802	mild	no	13.8	62	22	11	5	0	0	0	0	0	1307	11.6	-	microcytic tear drop	36.7	4.1	71.1	normocellular
47	914905	mild	no	22.3	86	10	3	1	0	0	0	0	0	657	12.9	-	normocytic	39.8	4.3	84.3	cellular
48	780561	mild	no	24	75	10	10	0	0	0	2	2	0	232	12.7	-	normocytic	31	3.99	86.2	hypercellular
49	538156	mild	no	69.7	58	1	3	2	1	0	25	6	4	236	9.6	present	normocytic	31.6	3.43	91.9	hypercellular
50	116967	mild	no	33	43	3	4	18	0	0	20	12	0	98	7.2	-	normocytic	22.9	2.14	86.8	hypercellular
51	625969	no	present	26.2	35	32	0	1	0	0	20	10	2	250	8.7	-	microcytic	29.8	3.66	81	hypercellular
52	687624	no	no	63	65	2	1	1	0	2	7	20	2	256	10.1	present	microcytic	32.5	4.01	70.6	hypercellular
53	712297	no	no	9.2	65	29	6	0	0	0	0	0	0	906	11.7	-	normocytic	40.1	5.4	80.3	dry tap
54	558937	massive	no	295	43	3	0	10	0	0	20	15	5	457	11.3	-	microcytic	34.7	4.29	67.1	hypercellular
55	557964	mild	present	5.9	84	15	1	0	0	0	0	0	0	172	8.4	present	microcytic tear drop cells	25.6	2.72	74.1	dry tap
56	456615	moderate	no	236	52	3	1	4	4	0	12	20	4	265	10.4	-	normocytic	38.6	4.1	83.4	hypercellular
57	578510	mild	no	40.8	72	12	5	3	0	0	5	2	1	271	13.6	-	normocytic	40.4	3.44	85.3	hypercellular
58	453845	moderate	no	43.9	68	13	5	2	0	0	8	4	0	272	11.8	present	normocytic	37	4.39	84.2	hypercellular
59	608482	moderate	no	245	66	4	2	4	2	4	10	8	0	275	10.8	-	normocytic	36	4.23	86.4	hypercellular
60	642490	no	no	74	56	5	2	2	2	1	15	13	4	291	7.4	present	microcytic	40	2.65	74.6	hypercellular
61	452833	moderate	no	102.5	52	8	2	3	1	0	11	23	0	294	10.5	-	normocytic	41.9	3.65	80.2	hypercellular
62	672654	moderate	no	86	50	6	0	2	2	2	23	12	3	296	13.1	-	normocytic	39.2	4.88	81.3	hypercellular
63	621139	no	no	42	79	6	2	1	0	0	5	5	2	298	12	present	normocytic	37.8	4.26	80.3	hypercellular
64	556268	moderate	no	114	60	2	0	2	2	0	20	10	2	506	10	present	macrocytic	42.1	3.98	93.1	hypercellular
65	238274	no	no	31.5	42	3	0	0	3	0	20	19	8	95	8.1	-	normocytic	43.1	3	84.3	normocellular
66	697511	mild	no	123.9	50	13	3	7	3	0	12	10	2	315	10	-	normocytic	34.6	3.67	80.7	hypercellular
67	715831	mild	no	18.6	38	15	2	10	4	2	15	12	2	338	13.9	-	normocytic	35.6	4.67	79.9	hypercellular
68	805503	moderate	no	135	87	3	2	0	0	0	4	4	0	341	12	present	normocytic	36.5	4.55	81.6	hypercellular
69	816910	no	no	144.6	53	6	0	3	2	0	15	16	5	342	11.3	present	normocytic	37.6	3.88	85.7	hypercellular
70	905162	mild	no	17.1	79	11	1	2	2	0	3	2	0	346	10	-	normocytic	38.9	3.66	84	hypercellular
71	932581	massive	no	334	46	5	3	7	1	0	25	10	3	348	10.1	present	microcytic	39.7	3.44	72.1	hypercellular
72	936634	massive	no	389	9	0	0	2	0	0	50	29	7	668	7	-	normocytic	40.3	2.46	84	hypercellular

		BONE MARROW ASPIRATION						
S.NO	MRD NO.	ERYTHEOPOISIS	LEUCOPOISIS	MEGAKARYOPOISIS	M:E	blasts	CELLULARITY	ERYTHROPOISIS
1	514845	normoblastic	myeloid hyperplasia with prominence of myelocytes and metamyelocytes	adequate	11:01	2	hypercellular	normoblastic
2	437885	depressed with normoblastic maturation	hyperplasia with bulge in myelocyte stage	prominent withdwarf forms	10:01	5	hypercellular	depressed
3	830523	depressed	hyperplasia	present with dwarf forms	12:01	0	hypercellular	normoblastic
4	848379	dry tap	-	-	-	10	hypercellular	depressed
5	514845	normoblastic	myeloid hyperplasia	hyperplasia	6.8:1	3	hypercellular	depressed
6	410487	prominence	prominence	hyperplasia with hypolobated forms	1:01	0	hypercellular	prominence
7	440037	-	-	-	-	0	hypocellular	depressed
8	464049	prominence	prominence	adequate with focal clustering	3.5:1	0	hypercellular	prominence
9	459637	normoblastic	orderly cell maturation	hyperplasia	3:01	-	hypercellular	normoblastic
10	416684	normoblastic with megaloblastic	hyperplasia with orderly cell maturation	adeqate with dwarf forms	10:01	0	hypercellular	normoblastic
11	478613	depressed normoblastic	hyperplasia with orderly cell maturation	present	13:01	2	hypercellular	depressed
12	502886	dry tap	-	-	-	-	hypercellular	depressed
13	518938	depressed	prominent orderly cell maturation	prominant	15:01	-	hypercellular	depressed
14	58732	few precursors	few precursors	few precursors	-	-	normocellular	normoblastic
15	540424	-	-	-	-	-	hypocellular	depressed
16	118749	normoblastic	depressed	prominant	2:01	-	hypercellular	normoblastic
17	604233	prominance	orderly cell maturation	prominent with young forms	-	0	hypercellular	normoblastic
18	610963	normoblastic	prominent orderly cell maturation	prominant	8:01ME	0blast	hypercellular	depressed
19	467195	normoblastic	orderly cell maturation	prominent with young forms	1.5:1	-	hypercellular	depressed
20	540259	normoblastic with megaloblastic	hyperplasia with orderly cell maturation	present	9:01	-	hypercellular	depressed
21	546682	normoblastic	hyperplasia with prominence of neutrophila	prominent	13:01	-	hypercellular	normoblastic
22	275365	depressed	myeloid hyperplasia	prominent with dwarf forms	12:01	2	hypercellular	depressed
23	396093	normoblastic	orderly cell maturation	prominent with hypolobated forms	2.3:1	-	normocellular	normoblastic
24	579606	normoblastic with megaloblastic	maturation arrest at blast stage	hyperplasia with atypical forms	-	40	hypercellular	depressed
25	635586	depressed with normoblastic maturation	hyperplasia with bulge in myelocyte stage	present	10:01	2	hypercellular	depressed
27	659530	depressed with normoblastic maturation	hyperplasia with bulge in myelocyte stage	prominent with dwarf forms	11:01	-	hypercellular	normoblastic
28	721990	normoblastic	myeloid prominence with hypogranular myelocytes	prominent	4.5:1	-	hypercellular	normoblastic
29	763211	normoblastic	myeloid hyperplasia with prominence of myelocytes and metamyelocytes	prominence	10:01	-	hypercellular	depressed
30	78114	depressed	myeloid hyperplasia with myelocyte and metamyelocyte bulge	prominence with dwarf forms	12:01	6	hypercellular	depressed
31	922657	normoblastic	hyperplasia with orderly cell maturation	occasional atypical forms	10:01	-	hypercellular	depressed
32	921895	normoblastic	hyperplasia	present	8:01	-	hypercellular	depressed
33	928854	depressed with normoblastic maturation	myeloid hyperplasia	prominence with dwarf forms	7:01	-	hypercellular	depressed
34	723056	normoblastic	orderly cell maturation	hyperplasia with atypical forms	3:01	-	hypercellular	normoblastic
35	723848	prominence with normoblastic and megaloblastoid change	-	hyperplasia	-	-	hypercellular	prominence
36	741476	normoblastic	prominenece	hyperplasia with atypical forms	-	-	hypercellular	normoblastic
37	605344	normoblastic	orderly cell maturation	prominent with atypical forms	-	-	hypercellular	normoblastic
38	483768	prominence with normoblastic and megaloblastoic change	orderly cell maturation	hyperplasia with atypical forms	-	-	hypercellular	prominence
39	838772	prominence	orderly cell maturation	hyperplasia with loose clustering and giant forms	1:01	-	hypercellular	prominence
39	766389	normoblastic	myeloid hyperplasia	prominent with dwarf forms	13:01	2	hypercellular	normoblastic
40	900201	depressed	myeloid hyperplasia	hyperplasia	9:01	-	hypercellular	depressed
40	864482	depressed with normoblastic maturation	hyperplasia	prominent	15:01	-	hypercellular	depressed
41	699164	depressed	myeloid hyperplasia	prominence with dwarf forms	13:01	-	hypercellular	depressed
42	920101	depressed	hyperplasia with orderly cell maturation	prominence	7:01	-	hypercellular	normoblastic
43	690257	depressed	hyperplasia	hyperplasia with dwarf forms	14:01	2	hypercellular	depressed
44	462539	normoblastic	hyperplasia with myelocyte and metamyelocyte bulge, blast2%	prominent with dwarf forms	13:01	-	hypercellular	depressed
45	632143	normoblastic	orderly cell maturation	hyperplasia with atypical forms	1:01	-	hypercellular	prominence
46	924802	normoblastic	orderly cell maturation	prominent	2.5:1	-	normocellular	normoblastic
47	914905	normoblastic	orderly cell maturation	hyperplasia with atypical forms	-	-	hypercellular	normoblastic
48	780561	depressed with normoblastic maturation	myeloid hyperplasia	prominence with dwarf forms	12:01	-	hypercellular	depressed
49	538156	depressed with normoblastic maturation	myeloid hyperplasia with prominence of myelocytes and metamyelocytes	prominence with dwarf froms	8:01	-	hypercellular	depressed
50	116967	normoblastic	hyperlasia with prominence of eosinophils	adequate	9:01	-	hypercellular	normoblastic
51	625969	depressed with normoblastic	prominence	prominent	10:01	-	hypercellular	depressed
52	687624	depressed	myeloid hyperplasia	hyperplasia with dwarf forms	6:01	-	hypercellular	depressed
53	712297	-	-	-	-	-	hypercellular	depressed
54	558937	normoblastic	prominence	prominent	10:01	-	hypercellular	normoblastic
55	557964	-	-	-	-	-	hypocellular	depressed
56	456615	depressed	hyperplasia	prominent	15:01	-	hypercellular	depressed
57	578510	depressed	prominence with orderly cell maturation	prominent with atypical forms	13:01	-	hypercellular	depressed
58	453845	normoblastic	hyperplasia	hyperplasia	6:01	-	hypercellular	normoblastic
59	608482	depressed	myeloid hyperplasia	hyperplasia with dwarf forms	12:01	4	hypercellular	depressed
60	642490	depressed with normoblastic maturation	myeloid hyperplasia	prominent	12:01	2	hypercellular	depressed
61	452833	depressed	myeloid hyperplasia	prominence with dwarf forms	7:01	-	hypercellular	depressed
62	672654	depressed	myeloid hyperplasia	hyperplasia with clustering and dwarf froms	12:01	3	hypercellular	depressed
63	621139	normoblastic	hyperplasia	adequate	7:01	-	hypercellular	normoblastic
64	556268	relatively depressed with normoblastic	hyperplasia with bulge in myelocyte stage	hyperplasia with atypical forms	12:01	-	hypercellular	depressed
65	238274	normoblastic	hyperplasia	prominant	8:01	12 blast	hypercellular	normoblastic
66	697511	relatively depressed with normoblastic	hyperplasia with bulge in myelocyte stage	prominent with atypical forms	12:01	3	hypercellular	normoblastic
67	715831	normoblastic	orderly cell maturation with 50% eosinophilic precursors	normal in number and morphology	2.3:1	-	hypercellular	normoblastic
68	805503	normoblastic	hyperplasia	prominent	13:01	-	hypercellular	normoblastic
69	816910	normoblastic	hyperplasia with orderly cell maturation	prominent with atypical forms	8:01	2	hypercellular	normoblastic
70	905162	normoblastic	hyperplasia with orderly cell maturation	hyperplasia with hypolobated forms	10:01	-	hypercellular	normoblastic
71	932581	depressed	hyperplasia	hyperplasia with atypical forms	13:01	-	hypercellular	depressed
72	936634	normoblastic	prominence	focal clustering	12:01	6	hypercellular	normoblastic

		BONE MARROW BIOPSY			DIAGNOSIS				
S.NO	MRD NO.	MYELOPOISIS	MEGAKARYOPOISIS	RETICULIN	GENETIC	FINAL	TECHNIQUE	RESULT	KARYOTYPING
1	514845	hyperplasia	hyperplasia with dwarf forms	grade1	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR TRANSCRIPT	-
2	437885	hyperplasia	hyperplasia with dwarf forms	grade2	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR TRANSCRIPT	-
3	830523	hyperplasia	hyperplasia with dwarf forms	no	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR TRANSCRIPT	-
4	848379	hyperplasia	prominence	grade1	bcr-abl+	CML in accelerated phase	RT-PCR quantitative	MAJOR AND MINOR TRANSCRIPT	-
5	514845	hyperplasia	prominence	grade1	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR TRANSCRIPT	-
6	410487	orderly cell maturation	hyperplasia with atypical forms	no	jak2+	polycythemia vera	RT-PCR quantitative	jak2v617f+	-
7	440037	depressed	depressed	grade3	bcr-abl-, jak2+	primary myelofibrosis	RT-PCR quantitative	jak2v617f+	present
8	464049	hyperplasia	adequate with focal clustering	no	bcr-abl- jakv617f+	polycythemia vera	RT-PCR quantitative	jak2v617f+	-
9	459637	orderly cell maturation	hyperplasia with clustering and atypical forms	grade1	bcr-abl- jak2+	ET	RT-PCR quantitative	jak2v617f+	-
10	416684	hyperplasia	hyperplasia with atypical forms	no	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR TRANSCRIPT	present del11q13q23
11	478613	hyperplasia	hyperplasia with dwarf forms	grade2	bcr-abl+	CML Chronic phase	FISH	bcr-abl+	-
12	502886	depressed	hyperplasia with clustering	grade3	bcr-abl- jak+	primary myelofibrosis	RT-PCR qualitative	jak2v617f+	-
13	518938	hyperplasia	hyperplasia with atypical forms	grade1	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR TRANSCRIPT	-
14	58732	orderly cell maturation	hyperplasia with clustering	no	jak2+	ET	RT-PCR quantitative	jak2v617f+	-
15	540424	depressed	hyperplasia with clustering	grade1	jak2 v617f+	primary myelofibrosis	RT-PCR qualitative	jak2v617f+	present
16	118749	depressed	prominence	grade1	jak2v617f+	primary myelofibrosis	RT-PCR qualitative	jak2v617f+	-
17	604233	orderly cell maturation	hyperplasia with clustering	grade1	jak2 exon14+ bcr-abl/calr/mpl-	primary myelofibrosis	RT-PCR qualitative	jak2v617f+	present
18	610963	hyperplasia	hyperplasia with atypical forms	grade2	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR TRANSCRIPT	present
19	467195	depressed	hyperplasia with clustering	grade2	jak2+	primary myelofibrosis	FISH	jak2v617f+	-
20	540259	hyperplasia	adequate	no	bcr-abl+	CML Chronic phase	RT-PCR qualitative	MAJOR TRANSCRIPT	-
21	546682	hyperplasia	prominence	grade1	jak2+	primary myelofibrosis	RT-PCR qualitative	jak2v617f+	-
22	275365	hyperplasia	adequate	grade1	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR TRANSCRIPT	-
23	396093	orderly cell maturation	prominence	no	jak2+	ET	RT-PCR qualitative	jak2v617f+	present
24	579606	hyperplasia	prominence	grade1	bcr-abl+	CML in blast crisis	RT-PCR quantitative	MAJOR TRANSCRIPT	-
25	635586	hyperplasia	prominence	no	bcr-abl+	CML Chronic phase	RT-PCR qualitative	MAJOR AND MINOR TRANSCRIPT	-
27	659530	hyperplasia with prominence eosinophilic precursors	hyperplasia with dwarf forms	no	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR TRANSCRIPT	-
28	721990	hyperplasia	prominence	no	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR TRANSCRIPT	-
29	763211	hyperplasia	prominence	no	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR TRANSCRIPT	-
30	78114	hyperplasia	hyperplasia with clustering	grade3	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR TRANSCRIPT	-
31	922657	hyperplasia	adequate	grade2	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR TRANSCRIPT	-
32	921895	hyperplasia	prominence	grade2	bcr-abl+	CML Chronic phase	RT-PCR qualitative	MAJOR TRANSCRIPT	-
33	928854	hyperplasia	hyperplasia with dwarf forms	no	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR TRANSCRIPT	-
34	723056	orderly cell maturation	hyperplasia with atypical forms	no	jak2+	ET	RT-PCR qualitative	jak2v617f+	-
35	723848	hyperplasia	prominence	no	jak2v617f+	polycythemia vera	RT-PCR qualitative	jak2v617f+	present
36	741476	orderly cell maturation	hyperplasia with atypical forms	no	jak2+	ET	RT-PCR qualitative	jak2v617f+	-
37	605344	hyperplasia	hyperplasia with atypical forms	no	jak2+	polycythemia vera	RT-PCR qualitative	jak2v617f+	-
38	483768	orderly cell maturation	hyperplasia with atypical forms	no	jak2+	polycythemia vera	RT-PCR qualitative	jak2v617f+	present
39	838772	hyperplasia	hyperplasia with clustering	grade1	jak2+	polycythemia vera	RT-PCR qualitative	jak2v617f+	-
39	766389	hyperplasia	hyperplasia with dwarf forms	no	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR TRANSCRIPT	-
40	900201	hyperplasia	prominence	no	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR AND MINOR TRANSCRIPT	-
40	864482	hyperplasia	prominence	no	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR TRANSCRIPT	-
41	699164	hyperplasia	hyperplasia with dwarf forms	no	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR TRANSCRIPT	-
42	920101	hyperplasia	hyperplasia with clustering	grade2	bcr-abl+	CML Chronic phase	FISH	bcr-abl+	-
43	690257	hyperplasia	hyperplasia with dwarf forms	grade1	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR TRANSCRIPT	-
44	462539	hyperplasia	hyperplasia with dwarf forms	grade1	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR TRANSCRIPT	-
45	632143	orderly cell maturation	hyperplasia with atypical forms	no	jak2+	polycythemia vera	RT-PCR qualitative	jak2v617f+	-
46	924802	orderly cell maturation	prominence	no	jak2 v617f+	ET	RT-PCR qualitative	jak2v617f+	-
47	914905	orderly cell maturation	hyperplasia with clustering	no	Jak2+	ET	RT-PCR qualitative	jak2v617f+	-
48	780561	hyperplasia	adequate	no	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR AND MINOR TRANSCRIPT	-
49	538156	hyperplasia	hyperplasia with dwarf forms	grade2	bcr-abl+	CML Chronic phase	FISH	bcr-abl+	present
50	116967	hyperplasia with prominence eosinophilic precursors	adequate	no	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR TRANSCRIPT	-
51	625969	hyperplasia	hyperplasia with clustering	grade2	bcr-abl+	CML Chronic phase	FISH	bcr-abl+	-
52	687624	hyperplasia	hyperplasia with clustering	grade2	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR TRANSCRIPT	-
53	712297	depressed	hyperplasia with clustering	grade1	Jak2v617f+	ET	RT-PCR qualitative	jak2v617f+	-
54	558937	hyperplasia	hyperplasia with clustering	grade1	bcr-abl+	CML Chronic phase	RT-PCR qualitative	jak2v617f+	present
55	557964	depressed	adequate	grade3	bcr-abl- jak2+	primary myelofibrosis	RT-PCR qualitative	jak2v617f+	-
56	456615	hyperplasia	hyperplasia with clustering	grade1	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR TRANSCRIPT	-
57	578510	hyperplasia	hyperplasia with clustering	grade1	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR TRANSCRIPT	-
58	453845	hyperplasia	adequate	no	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR TRANSCRIPT	-
59	608482	hyperplasia	hyperplasia with clustering	grade2	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR TRANSCRIPT	-
60	642490	hyperplasia	hyperplasia with clustering	grade2	bcr-al+	CML Chronic phase	RT-PCR quantitative	MAJOR TRANSCRIPT	-
61	452833	hyperplasia	hyperplasia with dwarf forms	no	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR TRANSCRIPT	-
62	672654	hyperplasia	prominence	grade1	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR TRANSCRIPT	-
63	621139	hyperplasia	hyperplasia with atypical forms	no	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR TRANSCRIPT	-
64	556268	hyperplasia	hyperplasia with clustering	grade2	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR TRANSCRIPT	-
65	238274	hyperplasia	hyperplasia with atypical forms	no	bcr-abl+	CML in accelerated phase	RT-PCR quantitative	MAJOR TRANSCRIPT	-
66	697511	hyperplasia	hyperplasia with clustering	grade2	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR TRANSCRIPT	-
67	715831	hyperplasia with prominence eosinophilic precursors	adequate	no	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR TRANSCRIPT	-
68	805503	hyperplasia	prominence	grade1	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR TRANSCRIPT	-
69	816910	hyperplasia	hyperplasia with atypical forms	no	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR TRANSCRIPT	-
70	905162	hyperplasia	hyperplasia with dwarf forms	grade1	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR TRANSCRIPT	-
71	932581	hyperplasia	hyperplasia with atypical forms	grade2	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR TRANSCRIPT	-
72	936634	hyperplasia	hyperplasia with clustering	grade1	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR TRANSCRIPT	-

						CLINICAL HISTORY- chief complaints														GENERAL PHY	
S.NO	MRD NO.	HEMAT.NO	NAME	AGE	SEX	generalised weakness	malaise and fatigue	weight loss	fever	night sweats	abdominal pain	paresthesia	vertigo	headaches	dizziness	bleeding tendencies	bluish discolouration of digits	DURATION	PERSONAL HISTORY	Appearance	SPLEENOMEGALY
73	177072	20-471	PS	39	m	present	present	-	-	-	-	-	-	-	-	-	-	-	-	-	moderate
74	942281	20-492	RKG	55	m	present	-	-	-	-	-	-	-	-	-	-	-	10 days	-	pallor	moderate
75	957002	20-552	NM	51	m	present	-	present	-	-	-	-	-	-	-	-	-	-	-	pallor	massive
76	963837	20-584	MB	49	f	-	present	present	present	-	-	-	-	-	-	-	-	2months	desi medication intake	pallor	massive
77	970968	20-640	RK	34	f	present	present	present	-	-	-	-	-	-	-	-	-	-	-	-	moderate
78	97755	20-682	R	55	m	-	-	present	present	-	present	-	-	-	-	-	-	1 week	bipolar disorder	pallor	massive
79	980577	20-714	AS	50	m	present	present	present	-	-	-	-	-	-	-	-	-	2weeks	-	pallor	mild
80	905162	20-181	HK	29	f	-	-	-	present	-	-	-	-	-	-	-	-	-	-	pallor	no
81	975693	19-164	MK	25	f	present	-	-	-	-	-	-	-	-	-	-	-	-	-	-	moderate
82	982095	20-738	MJ	42	f	present	present	-	-	-	-	-	-	-	-	-	-	-	-	pallor	moderate
83	993938	20-805	S	27	f	-	present	-	present	present	-	-	-	-	-	-	-	-	-	-	mild
84	993878	20-806	K	43	f	present	-	-	-	-	-	-	-	-	-	-	-	-	-	-	mild
85	468705	18-289	KK	62	f	present	-	-	-	-	-	-	-	-	-	-	-	3weeks	DM postmenopausal	-	moderate
86	621139	18-1071	AK	43	m	present	-	-	-	present	-	-	-	-	-	-	-	1month	-	pallor	mild
88	1044727	21-175	PKG	50	m	-	present	-	-	-	-	-	-	-	-	-	-	-	-	-	moderate
89	1057768	21-217	HK	35	f	-	present	present	fever	-	-	-	-	-	-	-	-	-	-	-	massive
90	1080889	21-274	PK	46	f	present	-	-	-	-	-	-	-	-	-	-	-	2weeks	-	pallor	moderate
91	1104881	21-3907	MS	54	m	present	-	-	-	-	-	-	-	-	-	-	-	4weeks	-	-	moderate
92	1106104	21-338	A	35	f	-	-	-	present	-	present	-	-	-	-	-	-	-	-	pallor	mild
93	1107501	21-342	UR	41	f	present	-	-	-	present	-	-	-	-	-	-	-	5months	-	-	mild
94	378644	17-823	SK	38	f	-	present	-	present	-	-	-	-	-	-	-	-	-	-	-	no
95	372340	17-816	SS	30	m	present	present	present	-	-	-	-	-	-	-	-	-	-	-	-	mild
96	368894	17-780	RB	37	f	present	present	present	-	-	-	-	-	-	-	-	-	3months	-	pallor	moderate
97	362462	17-747	P	14	m	-	-	present	present	-	present	-	-	-	-	-	-	-	-	-	massive
98	361782	17-779	N	40	m	present	present	present	present	-	-	-	-	-	-	-	-	2months	scalp mass hpe-malignanct round cell tumor	-	mild
99	25665	17-683	SK	53	m	present	present	present	-	-	-	-	-	-	-	-	-	1month	H/O TB	-	mild
100	329595	17-614	ML	47	m	present	present	-	-	-	-	-	-	-	-	-	-	2weeks	-	pallor	moderate
101	354738	17-734	BS	65	m	present	-	-	-	-	-	present	-	-	-	-	present	4months	H/O TB	-	mild
102	298231	17-464	RS	48	m	present	-	-	-	-	-	-	-	-	-	-	-	3 months	-	pallor	moderate
103	72393	17-486	PD	54	f	present	-	-	present	-	-	-	-	-	-	-	-	20 days	-	pallor	massive
104	303103	17-479	JK	14	f	-	present	present	-	-	-	-	-	-	-	-	-	20days	-	pallor	moderate
105	276989	17-378	RPK	23	f	present	-	-	-	-	-	-	-	-	-	-	-	15days	-	-	moderate
106	236433	17-204	HS	65	m	present	-	present	present	-	-	-	-	-	-	-	-	5 months	chronic alcohol intake	pallor	massive
107	203343	17-96	SPS	61	m	present	-	-	present	-	-	-	-	-	-	-	-	-	-	pallor	moderate
108	216594	17-137	JS	42	m	-	present	-	present	-	-	-	-	-	-	-	-	15days	-	pallor	moderate
109	108989	16-356	JK	27	f	-	present	-	present	-	-	-	-	-	-	-	-	-	-	pallor	moderate
110	157997	16-572	SB	57	f	present	present	-	-	present	-	-	-	-	-	-	-	1month	DM HTN	-	moderate
111	117507	16-430	LD	70	f	present	present	-	-	-	-	-	-	-	-	-	-	1month	DM HTN	pallor	moderate
112	163407	16-499	MT	54	m	present	-	present	-	-	-	-	-	-	-	-	-	10days	-	pallor	mild
113	154633	16-905	HS	40	m	present	-	present	-	-	-	-	-	-	-	-	-	5days	alcoholic	pallor	massive
114	58796	16-449	SR	68	f	present	-	-	-	present	-	-	-	-	-	-	-	2months	HTN	pallor	moderate
115	108516	16-357	MS	48	f	present	present	-	present	-	-	-	-	-	-	-	-	2weeks	DM	-	mild
116	82892	16-260	RK	40	m	-	present	present	present	-	present	-	-	-	-	-	-	15days	-	-	moderate
117	78329	16-516	BK	70	f	-	-	-	-	-	-	present	-	present	present	-	-	5 month	-	pallor	mild
118	137518	16-628	AK	50	m	present	present	-	present	-	-	-	-	-	-	-	-	15days	-	pallor	moderate
119	128508	16-795	ND	50	f	present	present	-	-	-	-	-	-	-	-	present	-	1month	-	pallor	moderate
120	607763	16-941	P	30	m	present	present	-	-	-	-	-	-	-	-	-	-	4weeks	-	pallor	moderate
121	407230	16-378	AS	59	m	present	-	-	present	present	-	-	-	-	-	-	-	20days	DM HTN	-	massive
122	120990	16-458	SK	63	m	present	present	-	-	-	-	-	-	-	-	-	-	7 days	alcoholic	-	no
123	125864	16-805	GK	55	f	present	-	present	-	-	-	-	present	-	-	-	-	1month	epileptic	-	mild
124	23598	16-739	BK	48	f	present	-	-	-	present	-	-	-	-	-	-	-	3weeks	-	pallor	no
125	74632	16-493	AK	23	f	present	-	-	-	-	-	-	-	-	-	-	-	4 days	-	-	moderate
126	99064	16-321	KB	50	f	present	-	present	present	-	-	-	-	-	-	-	-	3months	-	pallor	mild
127	1015160	21-49	GS	71	m	present	-	-	-	present	-	-	-	-	-	-	-	2 months	-	pallor	mild
128	1011426	21-29	PK	44	m	-	-	present	present	-	-	-	-	-	-	-	-	1month	-	pallor	moderate
129	992145	21-02	AK	13	f	-	present	-	-	-	-	-	-	-	-	-	-	10days	-	-	mild
130	1029683	21-126	HK	70	f	-	present	-	present	present	present	-	-	-	-	-	-	6 month	desi medication intake	-	moderate
131	1022809	21-91	JS	28	m	-	present	-	-	-	-	-	-	-	-	-	-	-	-	-	mild
132	1069548	21-240	ML	65	m	-	-	-	-	-	-	-	present	present	present	-	-	4 month	smoking and alcohol intake	-	moderate
133	1062966	21-231	PS	65	m	present	present	present	-	-	-	-	-	-	-	-	-	4.5months	-	-	moderate
134	195589	21-709	SK	63	f	present	-	-	present	-	-	-	-	-	-	-	-	2months	-	pallor	no
135	452589	18-261	AKS	47	m	-	present	present	-	-	-	-	-	-	-	-	-	1month	jaundice	-	mild
136	1035915	17-896	JS	40	m	-	present	present	-	-	-	-	-	-	-	-	-	1month	-	pallor	mild
137	627589	19-41	RK	55	f	present	-	present	-	-	-	-	-	-	-	present	-	2 month	-	-	no
138	443454	20-21	KKA	74	m	present	present	present	-	-	-	-	-	-	-	-	-	1month	reformed alcoholic	pallor	no

		SICAL EXAMINATION		HEMATOLOGICAL PARAMETERS																	
S.NO	MRD NO.	HEPATOMEGALY	lymphadenopathy	TLC	neutrophil	lymphocyte	monocyte	eosinophil	basophil	promyelocyte	myelocyte	metamyelocyte	blast	PLATELETS	HG g/dl	nrbcs	PBF	hematocrit	rbc count	MCV	CELLULARITY
73	177072	moderate	no	71.7	64	7	0	5	5	0	10	7	2	361	11.9	present	normocytic	41.6	3.99	80	hypercellular
74	942281	mild	no	131	90	3	5	0	0	0	1	1	0	370	8.6	-	normocytic	42.8	4.01	81	hypercellular
75	957002	massive	no	253.2	46	4	26	4	2	0	6	10	2	376	10.7	present	microcytic	43.7	4.11	74	hypercellular
76	963837	moderate	no	147	40	8	0	3	2	0	30	16	1	378	11.8	-	normocytic	36.8	4.22	86	hypercellular
77	970968	no	no	147	40	8	0	3	2	0	20	26	1	378	11.8	-	normocytic	35.7	4.33	86.7	hypercellular
78	97755	massive	no	530	43	3	1	7	0	0	20	18	8	385	7.3	present	normocytic	34.9	2.9	87.1	hypercellular
79	980577	mild	no	55.4	63	11	0	3	3	0	10	7	3	385	9.6	present	macrocytic	33.6	3.9	90.6	hypercellular
80	905162	moderate	no	42.3	95	4	1	0	0	0	0	0	0	411	8.1	-	normocytic	34.8	3.22	87.1	hypercellular
81	975693	no	no	91.2	49	2	0	5	2	4	20	14	4	414	12.6	present	normocytic	33.7	3.88	81	hypercellular
82	982095	moderate	no	294	50	2	15	1	0	0	15	15	2	418	10.6	present	macrocytic	32.1	3.64	89.9	hypercellular
83	993938	mild	no	75.5	78	4	1	1	0	0	10	5	1	427	13	-	normocytic	30.2	4.01	87	hypercellular
84	993878	moderate	no	125	40	0	0	6	0	2	23	10	9	438	13.2	-	normocytic	34.6	4.11	79.8	hypercellular
85	468705	mild	no	141	44	2	0	3	5	0	25	15	6	439	15.9	-	normocytic	38.9	4.65	86	hypercellular
86	621139	no	no	83	75	5	5	0	0	0	10	5	0	450	9.9	present	normocytic	39.9	4.11	84.2	hypercellular
88	1044727	mild	no	121.3	35	2	1	10	2	0	20	8	2	455	12.5	present	normocytic	40.6	4.1	81.2	hypercellular
89	1057768	moderate	no	295	43	3	0	10	0	0	20	15	5	457	11.3	-	normocytic	42.8	3.89	82.1	hypercellular
90	1080889	moderate	no	154.4	56	3	0	0	3	2	17	10	9	480	9.3	-	normocytic	40.9	3.54	80.2	hypercellular
91	1104881	mild	no	140	47	2	0	6	3	0	20	17	5	500	10.7	present	normocytic	41.3	3.54	81.2	hypercellular
92	1106104	no	no	82	64	5	0	3	3	0	10	10	5	512	9.9	-	normocytic	40.9	3.65	80.6	hypercellular
93	1107501	no	no	73	62	3	0	2	0	2	15	15	0	526	11.6	present	normocytic	38.6	4.01	80.3	hypercellular
94	378644	mild	no	46	60	5	2	2	0	2	17	10	2	540	11.7	present	normocytic	32.9	3.26	82.6	hypercellular
95	372340	no	no	179	46	2	0	0	2	0	20	25	5	210	10.1	present	normocytic	30.8	3.11	98.9	hypercellular
96	368894	no	no	14.9	75	22	1	2	0	0	0	0	0	896	13.2	-	normocytic	41.8	4.53	92.2	hypercellular
97	362462	mild	no	302.9	40	2	1	2	4	0	22	27	2	558	2.7	present	normocytic	6.9	0.95	83	hypercellular
98	361782	no	no	130.7	60	5	1	5	3	0	13	10	3	809	12.8	present	normocytic	37.1	4.3	86.1	hypercellular
99	25665	no	present	96	52	1	1	2	1	2	30	10	1	568	11.3	present	normocytic	35.4	3.57	99.1	hypercellular
100	329595	mild	no	125	60	4	0	2	1	0	20	10	3	572	10.9	-	normocytic	40.8	4.54	89.7	hypercellular
101	354738	mild	present	15	87	7	3	3	0	0	0	0	0	1073	14.7	-	normocytic	48.6	7.93	90.1	hypercellular
102	298231	mild	present	168.5	75	1	2	0	2	0	15	5	0	579	10.6	present	microcytic	41.3	5.02	72.2	hypercellular
103	72393	moderate	no	329	40	0	1	9	7	3	12	23	7	584	8.8	present	normocytic	31	3.59	86.4	hypercellular
104	303103	mild	no	253.2	67	5	0	0	2	4	15	5	2	590	14.1	present	normocytic	30.4	3.58	85.1	hypercellular
105	276989	no	no	258	52	7	0	4	4	0	14	20	4	593	10.6	present	normocytic	35.7	3.2	86.1	hypercellular
106	236433	no	no	31.5	72	3	5	6	0	0	4	10	0	249	9.5	present	microcytic tear drop cells	33.2	5.33	60.4	scanty fragments,
107	203343	moderate	no	301.7	44	2	1	0	4	0	12	30	7	650	8.3	present	normocytic	41.6	4.79	86.9	hypercellular
108	216594	moderate	no	287.6	54	10	3	6	0	0	10	15	3	671	9.9	present	microcytic	29.8	3.2	72.9	hypercellular
109	108989	moderate	no	178.1	52	7	0	4	4	0	14	20	4	704	8.1	-	normocytic	25.4	2.98	85.3	hypercellular
110	157997	moderate	no	130.7	60	5	1	5	3	0	13	10	3	406	12.8	present	normocytic	36.3	3.51	83.3	hypercellular
111	117507	mild	no	49.6	74	9	5	2	0	0	4	4	2	420	9.22	present	microcytic	26.5	3.44	77.2	hypercellular
112	163407	mild	no	92	66	8	2	4	1	0	10	7	2	348	10.1	-	normocytic	32.3	3.79	85.3	hypercellular
113	154633	moderate	no	126	59	11	0	3	1	0	14	10	2	433	9.2	present	normocytic	40.1	4.37	91.7	hypercellular
114	58796	mild	no	144.8	65	2	1	1	0	2	7	20	2	346	10	present	microcytic	32.1	3.98	80.6	hypercellular
115	108516	mild	no	9.1	77	14	8	1	0	0	0	0	0	1549	13.8	-	normocytic	43	5.17	83.2	hypercellular
116	82892	massive	no	301.7	44	2	1	0	4	0	20	22	7	650	8.3	present	normocytic	24.2	2.71	89.2	hypercellular
117	78329	no	no	20.5	65	30	4	2	0	0	0	0	0	619	17.6	-	normocytic	53.7	7	79.8	hypercellular
118	137518	mild	no	47.7	78	2	4	0	3	0	8	4	1	512	9.9	-	normocytic	31.8	3.68	86.3	hypercellular
119	128508	moderate	no	132.7	44	3	0	3	4	0	23	20	3	102	8.7	present	normocytic	25.1	2.56	98	hypercellular
120	607763	moderate	no	123.9	50	13	3	7	3	0	11	11	2	315	10	present	normocytic	31.5	3.5	90.2	cellular
121	407230	moderate	no	114	75	9	0	2	2	0	6	4	2	306	14.1	present	microcytic	31	4.24	73.1	hypercellular
122	120990	mild	no	75.5	77	9	2	1	3	0	5	2	1	574	13	-	normocytic	40.8	4.35	93.8	hypercellular
123	125864	no	no	13.4	71	24	4	1	0	0	0	0	0	450	18	-	normocytic	58	9.07	64	normocellular
124	23598	mild	no	92.7	50	6	3	2	4	0	20	13	2	654	8.3	present	microcytic	28.5	3.96	72	hypercellular
125	74632	mild	no	54.5	73	8	4	0	0	0	10	5	0	479	13.1	-	normocytic	29.6	3.83	77.2	hypercellular
126	99064	moderate	no	154.4	56	3	0	0	3	2	10	17	9	541	9.8	-	normocytic	29.4	3.38	87	no fragments. Co
127	1015160	moderate	no	92.7	50	6	3	2	4	0	13	20	2	664	8.3	present	microcytic tear drop cells	32.6	4.01	65	hypercellular
128	1011426	no	no	141	24	6	3	8	1	2	25	29	2	450	6.9	-	macrocytic	40.2	4.23	92	hypercellular
129	992145	no	no	46	60	5	2	2	0	2	17	10	2	540	11.7	present	microcytic	35.8	4.33	67	hypercellular
130	1029683	mild	no	7.3	80	15	2	3	0	0	0	0	0	80	5.6	present	microcytic with tear drop cells	36.7	4.1	70.1	dry tap
131	1022809	massive	no	338	39	2	0	5	1	1	20	25	7	560	10.1	-	normocytic	42.6	3.54	84	hypercellular
132	1069548	mild	no	9.1	68	30	2	0	0	0	0	0	0	501	18.1	-	normocytic	49.1	8.99	87.9	hypercellular
133	1062966	no	no	8.7	62	28	8	2	0	0	0	0	0	336	18.3	-	normocytic	50.1	9.78	89.7	hypercellular
134	195589	mild	no	26.5	70	10	8	2	0	0	5	5	0	338	7.1	-	normocytic	44.5	2.98	86	hypercellular
135	452589	mild	no	11	76	16	5	3	0	0	0	0	0	477	20.3	-	normocytic	56.9	8.54	90.3	hypercellular
136	1035915	mild	no	18	71	20	3	2	0	0	2	2	0	918	8.5	present	microcytic	27.5	3.38	79.4	hypercellular
137	627589	mild	no	8.6	70	24	3	3	0	0	0	0	0	1033	11.3	present	microcytic	39.4	3.65	74.2	normocellular
138	443454	no	no	11.7	35	53	6	2	0	0	2	2	0	216	5.4	present	microcytic tear drop cells	43.2	3.54	68.9	hypercellular

		BONE MARROW ASPIRATION						
S.NO	MRD NO.	ERYTHEOPOISIS	LEUCOPOISIS	MEGAKARYOPOISIS	M:E	blasts	CELLULARITY	ERYTHROPOISIS
73	177072	depressed	hyperplasia	hyperplasia with atypical forms	12:01	-	hypercellular	normoblastic
74	942281	normoblastic	hyperplasia	present	8:01	-	hypercellular	normoblastic
75	957002	relatively depressed	hyperplasia with prominence with myelocyte and metamyelocyte	atypical forms	15:01	-	hypercellular	normoblastic
76	963837	depressed	myeloid hyperplasia	prominence	18:01	-	hypercellular	depressed
77	970968	depressed	myeloid hyperplasia	prominence	18:01	2	hypercellular	depressed
78	97755	depressed with normoblastic maturation	hyperplasia with myelocytes and metamyelocytes prominence and blasts7%	present with dwarf forms	15:01	-	hypercellular	depressed
79	980577	depressed with normoblastic maturation	hyperplasia with myelocyte metamyelocyte bulge	hyerplasia with atypical forms	8:01	5	hypercellular	depressed
80	905162	normoblastic	hyperplasia with orderly cell maturation	adequate	5:01	-	hypercellular	normoblastic
81	975693	depressed with normoblastic maturation	hyperplasia blasts6%	prominent with dwarf forms	-	-	hypercellular	depressed
82	982095	depressed	hyperplasia with bulge in myelocyte stage	present	12:01	1	hypercellular	depressed
83	993938	normoblastic	hyerplasia with orderly cell maturation	hyperplasia with hypolobated forms	9:01	-	hypercellular	normoblastic
84	993878	depressed	myeloid hyperplasia with myelocyte and metamyelocyte bulge	hyperplasia with dwarf froms	10:01	-	hypercellular	normoblastic
85	468705	depressed	myeloid hyperplasia with prominence of myelocytes and metamyelocytes	prominence with hypolobated forms	13:01	-	hypercellular	depressed
86	621139	depressed with normoblastic maturation	hyperplasia	hyperplasia with dwarf forms	9:01	-	hypercellular	depressed
88	1044727	normoblastic maturation	myeloid hyperplasia with prominence of eosinophilic precursors	prominence of dwarf forms	5:01	-	hypercellular	depressed
89	1057768	depressed with normoblastic maturation	myeloid hyperplasia	hyperplasia with dwarf forms	10:01	-	hypercellular	depressed
90	1080889	relatively depressed with normoblastic	prominence with bulge with myelocyte and metamyelocyte stage	prominent with atypical forms	-	6	hypercellular	depressed
91	1104881	depressed with normoblastic maturation	hyperplasia with orderly cell maturation	hyperplasia with dwarf forms	12:01	-	hypercellular	depressed
92	1106104	relatively depressed with normoblastic	hyperplasia with bulge in myelocyte stage	prominence with atypical forms	8:01	2	hypercellular	normoblastic
93	1107501	depressed	myeloid hyperplasia	hyperplasia with dwarf froms	12:01	-	hypercellular	normoblastic
94	378644	depressed with normoblastic maturation	hyperplasia with bulge in myelocyte stage	prominent with dwarf and atypical forms	12:01	1	hypercellular	depressed
95	372340	relatively depressed	hyperplasia with bulge in myelocyte stage	prominent	12:01	4	hypercellular	depressed
96	368894	normoblastic	orderly cell maturation	hyperplasia with hyperlobated forms	3.4:1	-	hypercellular	normoblastic
97	362462	normoblastic	hyperplasia with orderly cell maturation	hyperplasia with atypical forms	9:01	2	hypercellular	normoblastic
98	361782	depressed	hyperplasia with bulge in myelocyte stage	prominence with dwarf forms	11:01	4	hypercellular	depressed
99	25665	depressed with normoblastic maturation	myeloid hyperplasia with prominence of eosinophilic precursors	hyperplasia with dwarf forms and focal clustering	10:01	-	hypercellular	depressed
100	329595	depressed	myeloid hyperplasia	prominence with dwarf froms	12:01	1	hypercellular	depressed
101	354738	normoblastic	orderly cell maturation	hyperplasia	3.2:1	-	hypercellular	normoblastic
102	298231	normoblastic	hyperplasia	hyperplasia with dwarf forms	-	-	hypercellular	normoblastic
103	72393	depressed	marked hyperplasia	hyperplasia, dwarf	10:01	0:00	hypercellular	depressed
104	303103	depressed with normoblastic maturation	myeloid hyperplasia with myelocyte and metamyelocyte bulge	prominent with dwarf forms	11.5:1	-	hypercellular	depressed
105	276989	relatively depressed	myeloid prominance	prominence few atypical	18:01	-	hypercellular	depressed
106	236433	-	-	-	-	-	hypocellular	normoblastic
107	203343	depressed with normoblastic maturation	hyperplasia with bulge in myelocyte stage	prominence with hypolobated forms	15:01	2	hypercellular	depressed
108	216594	relatively depressed	hyperplasia with 4% blasts	hyperplasia with dwarf forms	-	-	hypercellular	depressed
109	108989	relatively depressed	hyperplasia	hyperplasia with atypical forms	18:01	4	hypercellular	depressed
110	157997	relatively depressed	hyperplasia with bulge in myelocyte stage	prominent	11:01	2	hypercellular	depressed
111	117507	normoblastic	orderly cell maturation	hyperplasia with dwarf forms	11:01	-	hypercellular	normoblastic
112	163407	normoblastic maturation	myeloid hyperplasia	hyperplasia with dwarf froms	9:01	-	hypercellular	normoblastic
113	154633	depressed with normoblastic maturation	hyperplasia with orderly cell maturation	hyperplasia with clustering and small megakaryocytes	15:01	-	hypercellular	depressed
114	58796	depressed with normoblastic maturation	myeloid hyperplasia	hyperplasia with dwarf forms	6:01	-	hypercellular	depressed
115	108516	normoblastic	orderly cell maturation	hyperplasia with normal morphology	2:01	-	normocellular	normoblastic
116	82892	normoblastic with megaloblastic	hyperplasia with bulge in myelocyte stage	hyperplasia with atypical forms	-	2	hypercellular	depressed
117	78329	normoblastic	orderly cell maturation	hyperplasia with clustering, hyper and hypolobated forms	1:01	2	hypercellular	prominence
118	137518	normoblastic	myeloid hyperplasia	hyperplasia with atypical forms	7:01	-	hypercellular	normoblastic
119	128508	normoblastic	hyperplasia with orderly cell maturation	hyperplasia with hypolobated and atypical forms	10:01	-	hypercellular	depressed
120	607763	depressed with normoblastic maturation	hyerplasia with blast8%	prominent with dwarf forms	12:01	3	hypercellular	depressed
121	407230	depressed with normoblastic maturation	prominence with orderly cell maturation	prominent with atypical forms	12:01	-	hypercellular	depressed
122	120990	normoblastic	hyperplasia with 4% blasts	hyperplasia with dwarf forms	6:01	-	hypercellular	normoblastic
123	125864	prominence with normoblastic maturation	orderly cell maturation	adequate	1:01	-	hypercellular	prominence
124	23598	normolastic	orderly cell maturation	hyperplasia with atypical forms and clustering	8:01	2	hypercellular	depressed
125	74632	normoblastic	hyperplasia	slightly prominent	5:01	-	hypercellular	normoblastic
126	99064	depressed with scattered erythrocyte precursors	orderly cell maturation	adequate	-	-	hypercellular	depressed
127	1015160	depressed	hyperplasia with orderly cell maturation	prominent with atypical forms	10:01	4	hypercellular	depressed
128	1011426	depressed with normoblastic	hyperplasia	prominence with atypical forms	11:01	2	hypercellular	depressed
129	992145	normoblastic	myeloid hyperplasia	prominence	10:01	-	hypercellular	normoblastic
130	1029683	-	-	-	-	-	hypocellular	depressed
131	1022809	depressed	myeloid hyperplasia	prominent with dwarf forms	11:01	-	hypercellular	depressed
132	1069548	normoblastic	orderly cell maturation	hyperplasia with clustering, hyper and hypolobated forms	2:01	-	hypercellular	normoblastic
133	1062966	prominence with normoblastic	orderly cell maturation	prominent with atypical forms	-	-	hypercellular	normoblastic
134	195589	depressed with normoblastic maturation	myeloid hyperplasia with orderly cell maturation	hyperplasia	11:01	-	hypercellular	depressed
135	452589	normoblastic	orderly cell maturation	hyperplasia with atypical forms	1.5:1	-	hypercellular	prominence
136	1035915	prominence with normoblastic and megaloblastoid change	orderly cell maturation	hyperplasia with atypical forms	1:01	-	hypercellular	hyperplasia
137	627589	normoblastic	orderly cell maturation	prominent	2:01	-	normocellular	normoblastic
138	443454	normoblastic with megaloblastic	hyperplasia with orderly cell maturation	prominent	4.5:1	-	hypercellular	normoblastic

		BONE MARROW BIOPSY			DIAGNOSIS				
S.NO	MRD NO.	MYELOPOISIS	MEGAKARYOPOISIS	RETICULIN	GENETIC	FINAL	TECHNIQUE	RESULT	KARYOTYPING
73	177072	hyperplasia	hyperplasia with clustering	grade1	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR TRANSCRIPT	-
74	942281	hyperplasia	adequate	no	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR TRANSCRIPT	-
75	957002	hyperplasia	hyperplasia with atypical forms	no	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR TRANSCRIPT	-
76	963837	hyperplasia	hyperplasia with atypical forms	no	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR TRANSCRIPT	-
77	970968	hyperplasia	hyperplasia with atypical forms	grade1	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR TRANSCRIPT	-
78	97755	hyperplasia	adequate	grade1	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR TRANSCRIPT	-
79	980577	hyperplasia	prominence	grade1	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR TRANSCRIPT	-
80	905162	hyperplasia	adequate	grade1	bcr-abl+	CML Chronic phase	RT-PCR qualitative	bcr-abl+	-
81	975693	hyperplasia	hyperplasia with dwarf forms	grade2	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR TRANSCRIPT	-
82	982095	hyperplasia	hyperplasia with atypical forms	no	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR TRANSCRIPT	-
83	993938	hyperplasia	prominence	grade1	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR TRANSCRIPT	-
84	993878	hyperplasia	hyperplasia with dwarf forms	no	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR TRANSCRIPT	-
85	468705	hyperplasia	hyperplasia with clustering	grade2	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR TRANSCRIPT	-
86	621139	hyperplasia	hyperplasia with dwarf forms	no	bcr-abl+	CML Chronic phase	RT-PCR qualitative	bcr-abl+	-
88	1044727	hyperplasia with prominence eosinophilic precursors	hyperplasia with dwarf forms	grade1	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR TRANSCRIPT	-
89	1057768	hyperplasia	hyperplasia with dwarf forms	grade1	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR TRANSCRIPT	-
90	1080889	hyperplasia	prominence	no	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR TRANSCRIPT	-
91	1104881	hyperplasia	prominence	grade1	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR TRANSCRIPT	-
92	1106104	hyperplasia	hyperplasia with atypical forms	grade1	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR TRANSCRIPT	-
93	1107501	hyperplasia	hyperplasia with dwarf forms	no	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR TRANSCRIPT	-
94	378644	hyperplasia	hyperplasia with dwarf forms	no	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR TRANSCRIPT	-
95	372340	hyperplasia	hyperplasia with clustering	grade1	bcr-abl+	CML Chronic phase	FISH	bcr-abl+	present
96	368894	orderly cell maturation	prominence	no	jak2+	ET	RT-PCR qualitative	jak2v617f+	-
97	362462	hyperplasia	hyperplasia with clustering	grade1	bcr-abl+	CML Chronic phase	FISH	bcr-abl+	-
98	361782	hyperplasia	hyperplasia with clustering	grade1	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR TRANSCRIPT	-
99	25665	hyperplasia	hyperplasia with dwarf forms	no	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR TRANSCRIPT	-
100	329595	hyperplasia	hyperplasia with dwarf forms	no	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR TRANSCRIPT	-
101	354738	orderly cell maturation	hyperplasia with clustering	no	jak2+	polycythemia vera	RT-PCR qualitative	jak2v617f+	-
102	298231	hyperplasia	prominence	no	bcr-abl+	CML Chronic phase	FISH	MAJOR TRANSCRIPT	-
103	72393	hyperplasia	hyperplasia with dwarf forms	no	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR TRANSCRIPT	-
104	303103	hyperplasia	hyperplasia with dwarf forms	no	bcr-abl+	CML Chronic phase	FISH	bcr-abl+	-
105	276989	hyperplasia	hyperplasia with atypical forms	grade2	bcr-abl+	CML Chronic phase	FISH	bcr-abl+	-
106	236433	orderly cell maturation	hyperplasia with clustering	grade3	bcr-abl- jak2+	primary myelofibrosis	RT-PCR qualitative	jak2v617f+	-
107	203343	hyperplasia	hyperplasia with clustering	grade1	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR TRANSCRIPT	-
108	216594	hyperplasia	hyperplasia with dwarf forms	grade1	bcr-abl+	CML Chronic phase	FISH	bcr-abl+	-
109	108989	hyperplasia	prominence	no	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR TRANSCRIPT	-
110	157997	hyperplasia	prominence	no	bcr-abl+	CML Chronic phase	RT-PCR qualitative	bcr-abl+	-
111	117507	hyperplasia with prominence eosinophilic precursors	prominence	no	bcr-abl+	CML Chronic phase	RT-PCR qualitative	bcr-abl+	-
112	163407	hyperplasia	hyperplasia with dwarf forms	grade1	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR TRANSCRIPT	-
113	154633	hyperplasia	prominence	no	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR TRANSCRIPT	-
114	58796	hyperplasia	hyperplasia with clustering	grade1	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR TRANSCRIPT	-
115	108516	orderly cell maturation	prominence	no	jak2+	ET	RT-PCR qualitative	jak2v617f+	-
116	82892	hyperplasia	hyperplasia with atypical forms	no	bcr-abl+	CML Chronic phase	RT-PCR qualitative	bcr-abl+	-
117	78329	orderly cell maturation	hyperplasia with clustering	no	jak2v617f+	polycythemia vera	RT-PCR qualitative	jak2v617f+	-
118	137518	hyperplasia	prominence	no	bcr-abl+	CML Chronic phase	FISH	bcr-abl+	-
119	128508	hyperplasia	prominence	no	bcr-abl+	CML Chronic phase	FISH	bcr-abl+	-
120	607763	hyperplasia	hyperplasia with clustering	grade3	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR AND MINOR TRANSCRIPT	-
121	407230	hyperplasia	prominence	no	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR TRANSCRIPT	-
122	120990	hyperplasia	hyperplasia with atypical forms	no	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR AND MINOR TRANSCRIPT	-
123	125864	orderly cell maturation	adequate	no	jak2+	polycythemia vera	RT-PCR quantitative	jak2v617f+	-
124	23598	hyperplasia	prominence	no	bcr-abl+	CML Chronic phase	FISH	bcr-abl+	-
125	74632	hyperplasia	prominence	no	bcr-abl+	CML Chronic phase	NGS panel	bcr-abl+	-
126	99064	hyperplasia	prominence	grade2	bcr-abl+	CML Chronic phase	RT-PCR qualitative	bcr-abl+	-
127	1015160	hyperplasia	prominence	no	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR AND MINOR TRANSCRIPT	-
128	1011426	hyperplasia	hyperplasia with dwarf forms	grade1	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR TRANSCRIPT	-
129	992145	hyperplasia	prominence	no	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR AND MINOR TRANSCRIPT	-
130	1029683	depressed	depressed	grade2	jak2+	primary myelofibrosis	RT-PCR qualitative	jak2v617f+	-
131	1022809	hyperplasia with prominence eosinophilic precursors	hyperplasia with dwarf forms	no	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR AND MINOR TRANSCRIPT	-
132	1069548	orderly cell maturation	hyperplasia with clustering	grade1	jak2v617f+	polycythemia vera	RT-PCR qualitative	jak2v617f+	-
133	1062966	orderly cell maturation	hyperplasia with atypical forms	no	jak2+	polycythemia vera	RT-PCR qualitative	jak2v617f+	-
134	195589	hyperplasia	hyperplasia with atypical forms	no	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR AND MINOR TRANSCRIPT	-
135	452589	orderly cell maturation	hyperplasia with atypical forms	no	jakv617f+	polycythemia vera	RT-PCR qualitative	jak2v617f+	-
136	1035915	hyperplasia	hyperplasia with clustering	grade2	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR TRANSCRIPT	-
137	627589	hyperplasia	prominence	no	bcr-abl+	CML Chronic phase	RT-PCR quantitative	MAJOR TRANSCRIPT	-
138	443454	hyperplasia	prominence	no	bcr-abl+	CML Chronic phase	NGS panel	bcr-abl+	-

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The Principal,
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Subject: **Approval of Plan of Thesis.**

This is to inform you that the Faculty of Medical Seiences in its meeting held on 22.01.2021 has approved the plan of thesis of **Dr. Jasleen Kaur,** a student of your college for **MD Pathology** along with the names of Supervisor/Co-Supervisor as under:-

Topic Plan of Thesis
Hematological and genetic profile of myeloproliferative neoplasms

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The student has to submit the three copies of thesis (along with PDF format) , to the Controller of Examinations, BFUHS, Faridkot, at least six months before the commencement of theory examination, if he/she is otherwise eligible to take the examination. He /She must directed to attach a photocopy of this letter with the forwarding letter while submitting thesis to the University along with the letter of approval of University for change of supervisor/Co-Supervisor, change of topic,(if any).

Dr. S P Singh MS(Oph)
Controller of Examinations

Note :

1. ***If any discrepancy found regarding topic of thesis or Supervisor/Co-Supervisor, inform undersigned within one month after issue of this letter.***
2. *The student may submit the thesis as per the schedule already conveyed i.e for May/June examination 30th Nov of the preceding the year of examination and for Nov/Dec examinations 31st May of the year of examination.*

HEMATOLOGICAL AND GENETIC PROFILES OF MYELOPROLIFERATIVE NEOPLASMS

THESIS PROPOSAL

FOR APPROVAL OF SUBJECT OF THESIS TO BE SUBMITTED IN

PARTIAL FULFILMENT OF THE REQUIREMENTS

FOR THE DEGREE OF

MD

In

PATHOLOGY

OF THE

BABA FARID UNIVERSITY OF HEALTH SCIENCES

FARIDKOT

NOVEMBER, 2019

DR. JASLEEN KAUR

DEPARTMENT OF PATHOLOGY
DAYANAND MEDICAL COLLEGE AND HOSPITAL
LUDHIANA

ABSTRACT OF PLAN OF THESIS

Title	HEMATOLOGICAL AND GENETIC PROFILE OF MYELOPROLIFERATIVE NEOPLASMS
For the degree of	MD (PATHOLOGY)
Name of the candidate	JASLEEN KAUR
Supervisor	Dr Bhavna Malhotra, Professor, Department of Pathology, DMCH, Ludhiana
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University	Baba Farid University of Health Sciences, Faridkot

Introduction: Myeloproliferative neoplasms are characterized by chronic clonal expansion of hematopoietic precursor cells causing neoplastic proliferation of myeloid, erythroid and megakaryocytic lineage.

Aims & Objectives: To correlate the genetic profile with histopathopathological findings in patients with myeloproliferative neoplasms.

Material and Method: It will be a retrospective and prospective study of patients diagnosed with MPN

Clinical Significance: The evolution of molecular studies in myeloproliferative neoplasms (MPN) has enlightened us to understand the complexity of the disease. The peripheral blood picture of MPNs, especially CML, CNL, PV and ET, may mimic reactive conditions, thus identifying genetic cause central to the diagnosis of a clonal expansion and for exclusion of non-neoplastic disorders. Genetic studies has profound value in diagnosing myeloproliferative neoplasms and their response to therapy.

Keywords: Myeloproliferative neoplasms, Chronic myeloid leukemia, Philadelphia chromosome

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Candidate	Co-supervisor	Co-supervisor	Supervisor	Principal

CERTIFICATE OF FACILITIES AVAILABLE

This is to certify that the facilities for work on the subject of thesis titled **"HEMATOLOGICAL AND GENETIC PROFILE OF MYELOPROLIFERATIVE NEOPLASMS"** exist in the Department of Pathology, DMC&H, Ludhiana and will be provided to the candidate. We will see that the data being included in the thesis is genuine and is collected by the candidate herself under our supervision and guidance.

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APPROVAL PROFORMA
BY
RESEARCH & ETHICAL COMMITTEE
DAYANAND MEDICAL COLLEGE & HOSPITAL, LUDHIANA

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Department	Pathology
Topic of Thesis	HEMATOLOGICAL AND GENETIC PROFILE OF MYELOPROLIFERATIVE NEOPLASMS
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4	DATE OF ADMISSION OF COURSE	14th MAY.2018
5	TITLE OF THE TOPIC	Hematological and genetic profile of myeloproliferative neoplasms

6. BRIEF RESUME OF THE INTENDED WORK

6.1 NEED FOR THE STUDY

The understanding of genetic studies in myeloproliferative neoplasms has improved the understanding of this complex group of diseases. Integration of genomic data along with clinical, hematological, histological data [1] is essential to diagnosis, prognosis and monitoring of the disease progression and remission or relapse.

Current classification of myeloproliferative neoplasms is hampered by disease heterogeneity within, and clinical overlap between the subtypes. A genomic classification can identify patients with common causative biologic factors and this classification is stable over time, and is not dependent on blood-count thresholds levels for labeling the disease.[2]

Therefore, collective use of clinical data with genetic profiling can give a personalized predictive prognosis of an individual patient.[3] Such information empowers the clinician and support complex decision in choice and intensity of therapy. The development of myeloproliferative neoplasms is driven by mutations in BCR–ABL1 and JAK2 V617F, CALR, or MPL. Many patients also have additional drivers that include a wide range of other cancer genes. Each patient presents with different driver genetic mutations causing the neoplasm and thus variable phenotypic expression that is different clonal expansion. Driver mutations correlate with phenotype and prognosis.[3]

Thus complex genetic mutational variation is what contributes to heterogeneity in diagnostic features and outcomes in patients with myeloproliferative neoplasms.[3]

The major determining factor in treatment of patients with myeloproliferative neoplasms is predicted prognosis that is if the patient is expected to progress to leukemia or myelofibrosis of bone marrow, such patients will be candidates for intensive treatment. Such predictive prognosis is possible with genetic status of the patient which are clinically suspected or preliminary diagnosis made on bone marrow studies.[3]

Combining genomic data along with clinical data gives personalized predictions of patient's outcomes and supports the treatment of patients with myeloproliferative neoplasms. CML is defined by a molecular and cytogenetic abnormality, the *BCR-ABL1* fusion Cytogenetic and molecular evaluations in MPNs are, complementary and each of these mutations have a well-established role in the diagnosis and monitoring responses to therapy, particularly in CML.[1]

For example, the identification of the t(9;22)/*BCR-ABL1* fusion excludes the diagnosis of reactive processes that may mimic CML (e.g. leukemoid reaction), and other MPNs and MDS/MPNs that may present with absolute neutrophilia (e.g. CNL, PMF, CMML, atypical CML). Of note, t(9;22) is not specific for CML as it can be also identified in a subset of *de novo* B-lymphoblastic leukemia/lymphoma (up to 25% of cases in adults), mixed-phenotype acute leukemias, and *de novo* acute myeloid leukemia.[1]

Therefore the present study is being conducted to understand the morphological and genetic profiles of patients with MPN's which will help to diagnose these complex entities correctly and streamline their treatment.

6.2 REVIEW OF LITERATURE

Morphology remains the central distinguishing feature in the 2016 WHO system for classification of tumors of the hematopoietic and lymphoid tissues, although mutation screening is increasingly being utilized for confirmation of morphologic diagnosis and, at times, for directing the diagnostic process.[4]

The 2016 WHO category of MPNs includes the three major subcategories of JAK2/CALR/MPL mutationrelated MPNs (i.e., PV, ET, and PMF), as well as four other clinicopathologic entities: chronic myeloid leukemia (CML), chronic neutrophilic leukemia (CNL), chronic eosinophilic leukemia, not otherwise specified (CELNOS) and MPN, unclassifiable (MPN-U).

The combination of clinical, morphological, and molecular genetic features is thought by the WHO as the most suitable attempt to define disease entities such as MPNs.[4-6] Following the updated 2008 WHO classification[6], a number of clinical–pathological studies conducted by different groups have validated these diagnostic guidelines including the importance of morphological features.[7] However, a balanced and evidence based discussion concerning these diagnostic criteria persists.[8] In particular, it has been postulated that ET, PV, and PMF cannot be strictly discriminated by BM morphology as postulated by the WHO, owing to their mimicry to transform to each other.[9] It was argued that JAK2-mutated ET resembles PV for similarities of hematological presentation and incidence of clinical manifestations.

The 2008 WHO classification of MPN was largely inspired by the discovery of mutations in JAK2 (chr. 9p24), namely V617F [10] in exon 14 and indels in exon 12, and MPL (chr. 1p34), mainly at codon W515, that were incorporated as major diagnostic criteria.[6] The JAK2V617F is the most prevalent mutation in MPN, accounting for ~ 95% of PV and 60% of ET and PMF.

Variable deletions and insertions clustering at codon 537–543 in exon 12 of JAK2 are detected in ~ 3–5% of patients with JAK2V617F unmutated PV by using sensitive approaches, as mutation allelic burden in whole blood and purified granulocytes is low.[11] Mutations in

MPL cluster in exon 10 at codon 515, the most prevalent being a W to K, L, A, R transversion, and rarely at codon 505 (S > N), originally reported in familial cases of thrombocytosis.[12]

They are found in ET and PMF with approximate incidence of 4 and 8%.[13] Finally, in 2013, mutations in CALR (chr. 19p13.2), the gene encoding the endoplasmic reticulum-associated chaperone calreticulin, were detected in patients with JAK2/MPL unmutated ET and PMF.[14] These are highly heterogeneous indels, all clustering in exon 9 that encodes for the C-terminus portion of the protein. There are two prevalent (>80% of all CALR variants) mutation types, type 1 (a 52-bp deletion; p.L367fs*46) and type 2 (a 5-bp insertion; p. K385fs*47), whereas the remaining are defined as type 1- like and type 2-like based on predicted helix propensity similarities with the former.[15] The type 2 CALR mutations are preferentially associated with ET, whereas type 1 predominates in PMF. The above three driver mutations are listed as major criteria for PV (JAK2V617F and exon 12), ET, and PMF (JAK2V617F, CALR and MPL) in the revised 2016 classification.

Therefore, the modern diagnostic approach to MPN requires the knowledge of mutation status. However, in the instances when genotyping for these mutations is not available, or the mutations result absent in diagnostic samples, minor criteria in the WHO classification are included to support diagnosis otherwise.

For example, the diagnostic hallmark of CML is the invariable presence of the BCR-ABL1 mutation. However, minor BCRABL1- harboring sub-clones are sometimes detected in other myeloid neoplasms, including the JAK2/CALR/ MPL-mutated MPNs, and do not necessarily alter the morphologically prominent diagnosis. Similarly, JAK2- mutated clones are sometimes detected in patients with CML, especially after successful treatment with imatinib.[16]

Similarly, CNL constitutes clonal proliferation of mature neutrophils and is usually associated with activating mutations (mostly T618I) of the gene (CSF3R) encoding for the receptor for granulocyte colony-stimulating factor, also known as colony-stimulating factor 3. CSF3R mutations appear to be specific to WHO-defined CNL.[17] Diagnosis of

CNL requires exclusion other causes of neutrophilia, including infections and inflammatory processes, metastatic cancer, and plasma cell neoplasms with secondary neutrophilia.[18] Mature-appearing neutrophilia also occurs in other myeloid malignancies, including atypical CML, BCR-ABL1-negative (aCML) and chronic myelomonocytic leukemia.

The reproducibility of the histological characteristics as described in the WHO classification remains a debate issue. Although the overall histological evaluation shows a high degree of reproducibility, the identification of specific morphological features displays a more limited reproducibility among different hemato-pathologists. The level of consensus has revealed a wide range between 49 and 100% in some studies.[19] Several reasons acting alone or in concert may be hypothesized to account for these shortcomings: (1) failure to reproducibly identify standard BM features of distinctive diagnostic value undermining a correct morphological interpretation;[20,21]

(2) inclusion of small, non-representative biopsy specimens with extensive crushing artefacts or fragmentation;3) disregard of age-related adjustment for assessing hematopoietic cellularity; (4) inability of performing an ccurate fiber grading owing to a variety of staining artefacts; (5) unexperienced investigators.[19,22]

The WHO committee of hemato-pathologists, clinicians, and scientists with special interest in MPN has now delivered the most comprehensive and practically useful outline of diagnostic criteria for ET, PV, and PMF. The authors of the current review strongly recommend the collection of BM examination at time of diagnosis of MPN and encourage repeating the procedure during follow-up, in the presence of signs of progressive disease. In all instances, sufficient BM aspiration should be secured in order to allow screening for driver and other mutations, as well as cytogenetic analysis.

Special attention to morphology is required in order to distinguish ET from pre-PMF and JAK2-mutated ET from PV. Such details are prognostically relevant as survival has been shown to be the longest in

strictly WHO-defined ET, whereas it was significantly worse in pre-PMF and PV.

BM examination is also the most optimal method of obtaining cytogenetic information that has been shown to influence survival in both PMF and PV. Establishing driver mutational status in patients with MPN is not only important in complementing morphologic diagnosis but also provides important prognostic information.

In regards to diagnosis, PV is expected to be almost always accompanied by a JAK2 mutation, whereas the specific driver mutation cannot otherwise distinguish one MPN from another; however, in distinguishing ET frompre-PMF or mPV, a higher JAK2V617F allele burden favors the diagnoses of the latter rather than the former.

In terms of prognosis, thrombosis risk in ET is strongly tied to the presence of JAK2 mutations, whereas the presence of type 1/like CALR mutations in PMF portendssuperior survival. In the future, we expect an increasing role for other mutations in complementing morphologic diagnosis in MPN and providing additional prognostic information.

6.3 AIMS AND OBJECTIVES OF THE STUDY

To correlate the genetic profile with histopathopathological findings in patients with myeloproliferative neoplasms.

PROFORMA

Date: S.No.:

Name:

Age: Sex :

CR No.: DOA: DOD:

Address:

Phone no.:

Chief presenting complaints: **Duration**

Onset of symptoms:

Past history: **Duration**

Family history:

Personal history:

General Physical examination

Laboratory investigations

- Complete blood count: Hb. TLC, DLC, Platelets, PBF
- LAP Score,
- ESR
- Ultrasonography abdomen
- CT whole body/abdomen

Bone marrow examination:

Cellularity

Erythropoiesis

Leucopoiesis

Megakaryopoeisis

Other cells

Perls stain

Signature of candidate Signature of Supervisor

Bone marrow aspiration diagnosis:

Bone marrow biopsy:

Cellularity

Erythropoiesis

Myelopoiesis

Megakaryopoiesis

Reticulin

Masson trichrome

Morphological diagnosis

Molecular genetic findings:

Final diagnosis

Signature of candidate

Signature of Supervisor

7. MATERIALS AND METHODS

The present five year study 3.5 years retrospective (1st April 2016– 30th September 2019) and 1.5 years prospective (1st October 2019 – 31st March 2021) will be conducted in the Department of Pathology, Dayanand Medical College and Hospital. During this period all the genetically confirmed cases of myeloproliferative neoplasms will be included and their genetic and hematological profiles will be studied in detail by correlating the genetic profile with morphological findings of bone marrow aspiration and biopsy findings.

The detailed clinical history regarding the onset of symptoms and clinical progression of the disease process will be taken. Samples including bone marrow aspirations and bone marrow biopsy along with venous blood sample for complete blood counts will be obtained from patients after they have provided written informed consents.

7.1 Source of Data

All the patients of myeloproliferative neoplasms whose bone marrow aspirations and bone marrow biopsy has been done in the department of pathology and have been confirmed as myeloproliferative neoplasms by genetic analysis .

The genetic profile will be correlated with morphological findings.

7.2 METHOD OF COLLECTION OF DATA

INCLUSION CRITERIA

All the diagnosed cases of myeloproliferative neoplasms whose bone marrow aspirations and biopsy have been done in department of pathology.

EXCLUSION CRITERIA-

Other hematological conditions and malignancies.

Methodology: Bone marrow aspiration and biopsy will be performed in the pathology department of the referred patients. The bone marrow aspiration slides will be prepared and stained with giemsa and other cytochemical stains wherever required. Bone marrow biopsy will be kept in 10% formalin for fixation, which will be sectioned and slides will be prepared and stained with giemsa, hematoxylin and eosin (H&E) for

cell morphology, prussian blue stain for iron, reticulin stain and masson trichrome for fibrosis. In cases where need arises LAP score will be done. All these specimen will be sent for genetic testing and the confirmed cases of myeloproliferative neoplasms will be considered our study group.

DURATION OF STUDY

Retrospective: 3.5 years (1st April 2016– 30th September 2019)

Prospective: 1.5 years (1st October 2019 – 31st March 2021)

OUTCOME

All the findings of specimen will be documented according to the protocol.

Statistical Analysis

Data will be described in terms of range; mean ±standard deviation (± SD), frequencies (number of cases) and relative frequencies (percentages) as appropriate. All statistical calculations will be done using SPSS (Statistical Package for the Social Science) SPSS 21 version statistical program for Microsoft Windows.

7.3 DOES THE STUDY REQUIRE ANY INVESTIGATIONS OR INTERVENTIONS TO BE CONDUCTED ON PATIENTS OR OTHER HUMANS OR ANIMALS? IF SO, PLEASE DESCRIBE BRIEFLY.

No.

7.4 HAS ETHICAL CLEARANCE BEEN OBTAINED FROM YOUR INSTITUTION IN CASE OF 7.3?

Attached herewith

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INFORMED PATIENT CONSENT

Date: _____

I.....D/o, W/o, S/o.....am willing to participate in the study **"HEMATOLOGICAL AND GENETIC PROFILE OF MYELOPROLIFERATIVE NEOPLASMS"** The procedure with its complications has been explained to me in local vernacular language. I have fully understood that no special tests and extra expenditure in any form, merely for the purpose of thesis is being carried out. I am free to opt out the study any time without assigning any reason.

....

Signature of the Witness Signature of the patient

Name:

Signature of Candidate **Signature of Supervisor**

मरीज अनुमति फार्म

तिथि _____

मैं _____ पत्नी/पुत्र/पुत्री _____

निवासी

_____इस

शोधकार्य **"HEMATOLOGICAL AND GENETIC PROFILE OF MYELOPROLIFERATIVE NEOPLASMS"** में भाग लेने के लिए सहमत हूँ। मैं इस शोधकार्य में भाग लेने के लिए अपनी स्वेच्छा से तैयार हूँ। मैं पुष्टि करता/करती हूँ की यह स्वीकृति देने के लिए मुझे कोई आर्थिक प्रलोभन नहीं दिया गया और न ही दबाव डाला गया है। मुझे इस शोधकार्य का उद्देश्य स्पष्ट कर दिया गया है और मुझे यह विश्वास है की यह विज्ञान और मानवता के लाभ के लिए है। मुझे इस शोधकार्य में होने वाले जोखिम के बारे में समझा दिया गया है और मेरी समझ में यह जोखिम बहुत मामुली है।

मैं इस शोधकार्य में भाग लेने के सम्बन्ध में किसी भी समय अपनी अस्वीकृति व्यक्त कर सकता/सकती हूँ और मेरे अस्वीकृति व्यक्त करने का मेरे इलाज पर कोई प्रभाव नहीं पड़ेगा।

_____ _____

गवाह के हस्ताक्षर रोगी के हस्ताक्षर/अंगूठे का निशान

नाम: नाम:

_____

अन्वेषक के हस्ताक्षर

_____

विद्यार्थी के हस्ताक्षर

ਮਰੀਜ਼ ਰਜ਼ਾਮੰਦੀ ਫਾਰਮ

ਮਿਤੀ_____

ਮੈ_____ਪਤਨੀ/ਪੁੱਤਰ/ਪੁੱਤਰੀ_____

ਵਾਸੀ _____

"HEMATOLOGICAL AND GENETIC PROFILE OF MYELOPROLIFERATIVE NEOPLASMS" ਤੇ ਚਲ ਰਹੀ ਖੋਜ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਲਈ ਰਜ਼ਾਮੰਦ ਹਾਂ। ਇਸ ਖੋਜ ਕੰਮ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਲਈ ਮੈ ਆਪਣੀ ਮਰਜ਼ੀ ਨਾਲ ਤਿਆਰ ਹਾਂ। ਮੈ ਇਹ ਦਸਣਾ ਚਾਹੁੰਦਾ ਹਾਂ/ ਚਾਹੁੰਦੀ ਹਾਂ ਕੇ ਇਹ ਰਜ਼ਾਮੰਦੀ ਦੇਣ ਵਾਸਤੇ ਮੈਨੂ ਕਿਸੇ ਵੀ ਤਰਾਂ ਦਾ ਲੋਭ-ਲਾਲਚ ਨਹੀ ਦਿੱਤਾ ਗਿਆ ਅਤੇ ਨਾ ਹੀ ਕੋਈ ਦਬਾ ਪਾਇਆ ਗਿਆ ਹੈ। ਮੈਨੂ ਇਸ ਖੋਜ ਦਾ ਮਨੋਰਥ ਸਮਝਾ ਦਿੱਤਾ ਗਿਆ ਹੈ ਅਤੇ ਮੈਨੂ ਇਹ ਭਰੋਸਾ ਦਵਾਇਆ ਗਿਆ ਹੈ ਕੇ ਇਹ ਖੋਜ ਵਿਗਿਆਨ ਅਤੇ ਮਾਨਵਤਾ ਦੀ ਭਲਾਈ ਲਈ ਹੈ। ਮੈਨੂ ਇਸ ਖੋਜ ਵਿਚ ਹੋਣ ਵਾਲੇ ਖਤਰੇ ਬਾਰੇ ਸਮਝਾ ਦਿੱਤਾ ਗਿਆ ਹੈ ਅਤੇ ਮੈਨੂ ਇਹ ਯਕੀਨ ਹੈ ਕੇ ਇਹ ਖਤਰਾ ਬਹੁਤ ਮਾਮੂਲੀ ਹੈ। ਮੈਨੂ ਇਸ ਖੋਜ ਕੰਮ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਦਾ ਢੰਗ – ਤਰੀਕਾ ਸਮਝਾ ਦਿੱਤਾ ਗਿਆ ਹੈ।

ਮੈ ਇਸ ਖੋਜ ਕੰਮ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਬਾਰੇ ਆਪਣੀ ਰਜ਼ਾਮੰਦੀ ਕਿਸੇ ਵੀ ਸਮੇ ਵਾਪਸ ਲੈ ਸਕਦਾ ਹਾਂ/ ਸਕਦੀ ਹਾਂ ਅਤੇ ਮੇਰੀ ਰਜ਼ਾਮੰਦੀ ਵਾਪਸ ਲੈਣ ਦਾ ਮੇਰੇ ਇਲਾਜ ਤੇ ਕੋਈ ਅਸਰ ਨਹੀ ਹੋਵੇਗਾ।

_____ _____

ਗਵਾਹ ਦੇ ਹਸਤਾਖਰ ਮਰੀਜ਼ ਦੇ ਹਸਤਾਖਰ

ਮਿਤੀ_____ ਮਿਤੀ_____

ਪਤਾ _____ ਪਤਾ _____

_____

ਨਿਰੀਖਕ ਦੇ ਹਸਤਾਖਰ

_____

ਸਿਖਿਆਰਥੀ ਦੇ ਹਸਤਾਖਰ